A Dissertation on

"A COMPARATIVE ANALYSIS OF SERUM HOMOCYSTEINE LEVELS IN PRE ECLAMPSIA, ECLAMPSIA, ABRUPTIO PLACENTA AND NORMAL PREGNANCIES"

Dissertation Submitted to

THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY

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with partial fulfillment of the regulations

for the award of the degree of

M.S. (OBSTETRICS AND GYNAECOLOGY)

COIMBATORE MEDICAL COLLEGE

CHENNAI – 600 032

MAY - 2018

DECLARATION

I solemnly declare that this dissertation entitled **"A COMPARATIVE ANALYSIS OF SERUM HOMOCYSTEINE LEVELS IN PREECLAMPSIA, ECLAMPSIA, ABRUPTIO PLACENTA AND NORMAL PREGNANCIES"** is a bonafide and genuine research work done by me under the guidance and support of **Dr.R.Manonmani M.D., D.G.O.,** Head of the Department, Obstetrics and Gynaecology, Coimbatore Medical College Hospital.

Date: Signature of the Candidate

Place:

CERTIFICATE

This is to certify that the dissertation entitled **"A COMPARATIVE ANALYSIS OF SERUM HOMOCYSTEINE LEVELS IN PRE ECLAMPSIA, ECLAMPSIA, ABRUPTIO PLACENTA AND NORMAL PREGNANCIES"** is a bonafide and genuine research work done by **Dr. V.K.T ANNURADHA** in partial fulfillment of the requirement for the degree of M.S Obstetrics and Gynaecology under the guidance and support of **Dr.R.Manonmani M.D., D.G.O., Head of the Department, Obstetrics and Gynaecology,** Coimbatore Medical College Hospital, Coimbatore.

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Name of the Candidate:		**Dr. V.K.T. Annuradha**
Course	:	**MS (OG) Post Graduate**
Period of Study	:	**1 year**
College	:	**Coimbatore Medical College & Hospital.**
Dissertation Topic	:	**A comparative analysis of serum homocysteine levels in pre eclampsia,eclampsia,abruptio placenta and normal pregnancies**

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted and you are permitted to proceed with the above Study.

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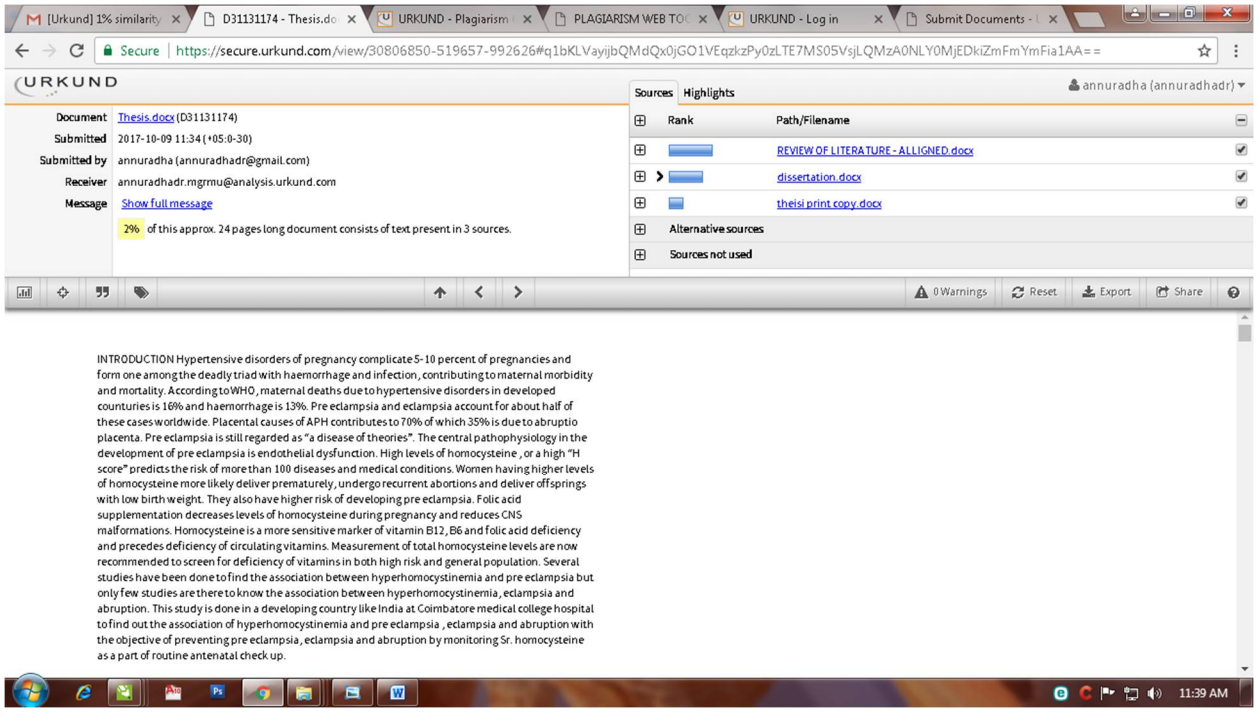
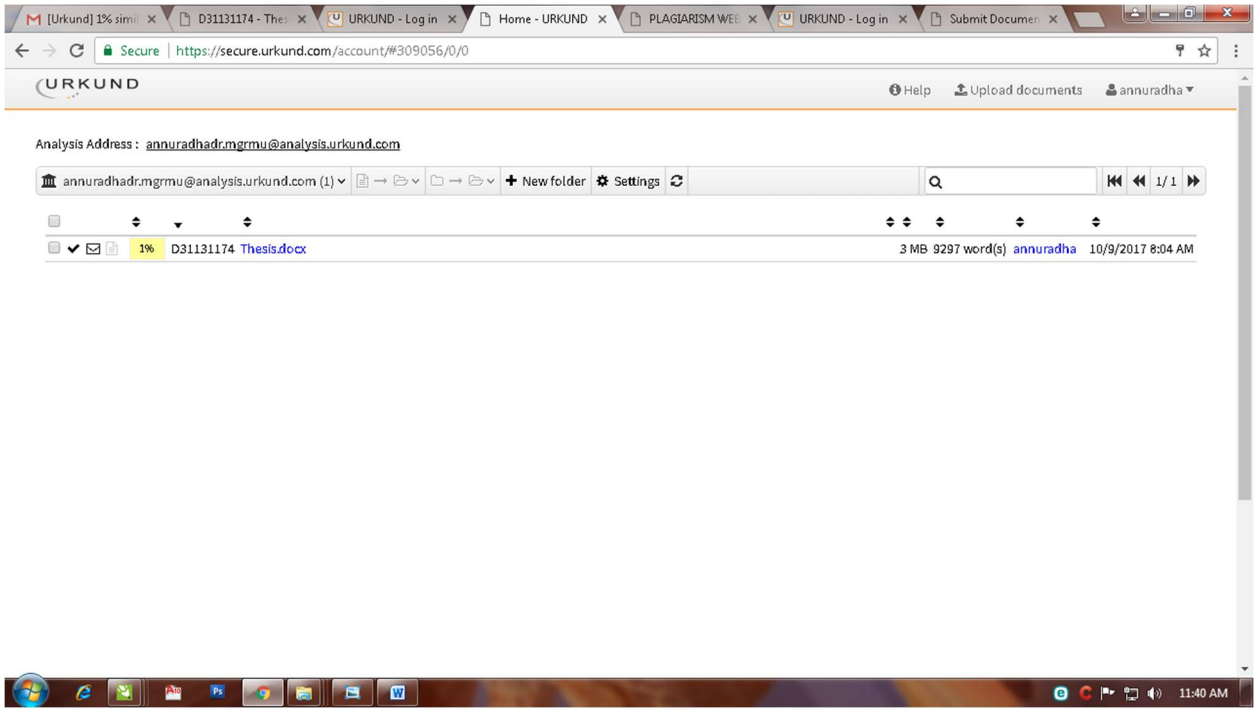


INTRODUCTION Hypertensive disorders of pregnancy complicate 5-10 percent of pregnancies and form one among the deadly triad with haemorrhage and infection, contributing to maternal morbidity and mortality. According to WHO, maternal deaths due to hypertensive disorders in developed counturies is 16% and haemorrhage is 13%. Pre eclampsia and eclampsia account for about half of these cases worldwide. Placental causes of APH contributes to 70% of which 35% is due to abruptio placenta. Pre eclampsia is still regarded as "a disease of theories". The central pathophysiology in the development of pre eclampsia is endothelial dysfunction. High levels of homocysteine , or a high "H score" predicts the risk of more than 100 diseases and medical conditions. Women having higher levels of homocysteine more likely deliver prematurely, undergo recurrent abortions and deliver offsprings with low birth weight. They also have higher risk of developing pre eclampsia. Folic acid supplementation decreases levels of homocysteine during pregnancy and reduces CNS malformations. Homocysteine is a more sensitive marker of vitamin B12, B6 and folic acid deficiency and precedes deficiency of circulating vitamins. Measurement of total homocysteine levels are now recommended to screen for deficiency of vitamins in both high risk and general population. Several studies have been done to find the association between hyperhomocystinemia and pre eclampsia but only few studies are there to know the association between hyperhomocystinemia, eclampsia and abruption. This study is done in a developing country like India at Coimbatore medical college hospital to find out the association of hyperhomocystinemia and pre eclampsia , eclampsia and abruption with the objective of preventing pre eclampsia, eclampsia and abruption by monitoring Sr. homocysteine as a part of routine antenatal check up.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INDEX

LIST OF TABLES

LIST OF GRAPHS

LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
BMI	Body mass index
BP	Blood Pressure
CBS	Cystathionine Beta Synthase
CRH	Corticotrophin Releasing Hormone
CVT	Cortical vein thrombosis
DIVC	Disseminated intravascular coagulation
DNA	Deoxy Ribo Nucleic Acid
GA	Gestational age
GFR	Glomerular filtration rate
HELLP	Hemolysis, Elevated Liver enzymes, Low Platelets
HLA	Human Leukocyte Antigen
IUGR	Intra Uterine Growth Restriction
LDL	Low Density Lipoprotein
LBW	Low Birth Weight
MHC	Major Histocompatibility Complex
MMP	Matrix Metallo Proteinase
MTHFR	Methylene Tetra Hydro Folate Reductase
PPH	Postpartum Haemorrhage
SAM	S Adenosyl Methionine
TNF	Tumour Necrosis Factor

INTRODUCTION

Hypertensive disorders of pregnancy complicate 5-10 percent of pregnancies and form one among the deadly triad with haemorrhage and infection, contributing to maternal morbidity and mortality. According to WHO, maternal deaths due to hypertensive disorders in developed countries is 16% and haemorrhage is 13%. Pre eclampsia and eclampsia account for about half of these cases worldwide.

Placental causes of APH contributes to 70% of which 35% is due to abruptio placenta.

Pre eclampsia is still regarded as “a disease of theories”. The central pathophysiology in the development of pre eclampsia is endothelial dysfunction.

High levels of homocysteine , or a high “H score” predicts the risk of more than 100 diseases and medical conditions.

Women having higher levels of homocysteine more likely deliver prematurely, undergo recurrent abortions and deliver offsprings with low birth weight. They also have higher risk of developing pre eclampsia.

Folic acid supplementation decreases levels of homocysteine during pregnancy and reduces CNS malformations.

Homocysteine is a more sensitive marker of vitamin B12, B6 and folic acid deficiency and precedes deficiency of circulating vitamins.

Measurement of total homocysteine levels are now recommended to screen for deficiency of vitamins in both high risk and general population.

Several studies have been done to find the association between hyperhomocysteinemia and pre eclampsia but only few studies are there to know the association between hyperhomocysteinemia, eclampsia and abruption.

This study is done in a developing country like India at Coimbatore medical college hospital to find out the association of hyperhomocysteinemia and pre eclampsia , eclampsia and abruption with the objective of preventing pre eclampsia, eclampsia and abruption by monitoring Sr. homocysteine as a part of routine antenatal check up.

AIMS AND OBJECTIVES

- To study the relationship between the levels of serum homocysteine in normal pregnancy and pregnancies complicated by pre eclampsia , eclampsia and abruption.
- To know, if the levels of serum homocysteine are indicators of severity of pre eclampsia, eclampsia and abruption, to reduce maternofetal morbidity and mortality.

REVIEW OF LITERATURE

Sunita Ghike et al studied serum homocysteine levels during normal pregnancy and pre eclampsia and concluded that homocysteine levels were reduced in 56% of pregnant normotensive women when compared to non pregnant women and levels were increased in 86% of women with pre eclampsia. Moreover serum homocysteine levels were more in women with severe pre eclampsia when compared to women with mild pre eclampsia[{1}] . This study shows that there is a link between increased serum homocysteine levels and pre eclampsia and its severity.

Md Mozammel Hoque et al studied serum homocysteine levels in pre eclampsia and eclampsia and concluded that in both pre eclampsia and eclampsia the levels of homocysteine were increased 9.54 ± 3.21 micromoles/litre and 10.57 ±3.39 micromoles/litre respectively[{2}]. From this study we infer that there is a significant increase in homocysteine levels in eclampsia in comparison to pre eclampsia.

Jian Wang et al studied elevated circulating homocysteine levels in placental vascular disease and associated pre eclampsia and infered that maternal homocysteine levels were increased in pre eclampsia and placental umbilical vascular disease but similar change was not seen in fetal homocysteine levels with placental vascular disease[{3}].This study shows that increase in circulating homocysteine levels might be a cause

for the pathogenesis of pre eclampsia due to uteroplacental vascular disorder.

Anderson A et al studied the levels of serum homocysteine in pregnancy and found that the total levels of serum homocysteine was very low in first trimester, it reached maximum in second trimester and the levels remained the same till delivery and returned back to normal in 2-4 days after delivery with P value < 0.05[4]. From this study we infer that homocysteine levels are decreased in normal pregnancies.

Vollset et al studied Plasma total homocysteine, pregnancy complications and adverse pregnancy outcomes and found that when homocysteine levels increased the risk of pre eclampsia was 32% more, for prematurity it was 38% more and for very low birth weight it was 101% more. There was significant association between plasma homocysteine levels, neural tube defects and club foot. But there was no relationship with abruptio placenta and homocysteine levels but when total homocysteine levels were >15micromoles/litre there was increased risk for abruption[5]. This study suggests a pivotal role of homocysteine in indicating pregnancy complications.

Seema Bibi Qureshi et al studied hyperhomocysteinaemia ,vascular related pregnancy complications and response to vitamin supplementation in pregnant women of Pakistan and concluded that preterm delivery and

low birth weight increased in women with pre eclampsia, eclampsia and abruptio placenta with P value < 0.001. Levels of homocysteine were considerably increased in pregnancies with pre eclampsia and eclampsia when compared to abruptio placenta and normal pregnant women. Moreover supplementation of folic acid, vitamin B6 and vitamin B12 showed a significant decrease in levels of homocysteine in previously elevated patients[6]. This study draws our attention to lower homocysteine levels by supplementation of folate, vitamin B6 and vitamin B12 in pregnant women with hyperhomocysteinemia.

Theresa O Scholl and William G Johnson studied the influence of folic acid on pregnancy outcome and found that deficiency of folate leads to hyperhomocysteinemia which is associated with recurrent abortions, pre eclampsia , abruptio placenta , preterm delivery and low birth weight[7] . This study insists on folic acid supplementation to prevent hyperhomocysteinemia.

Shilpa A.V. et al studied changes in homocysteine levels during normal pregnancy and pre eclampsia and its relation with oxidative stress and concluded that levels of serum homocysteine in pre eclampsia were lower compared to non pregnant normal woman with P value 0.001 but serum homocysteine levels were higher in pre eclampsia when compared to pregnant women without any complications but it was not significant

statistically with P value 0.677. This shows that folate intake in first trimester in this study might be the cause for minimum increase in homocysteine levels compared to pregnant women with no complications. The product of lipid peroxidation – malondialdehyde levels were measured which showed that in pre eclampsia the levels were considerably increased and statistically significant when compared to normal non pregnant and pregnant women with P value 0.001[8]. This study shows a positive association between homocysteine levels and MDA levels in pre eclamptics and infers the role of oxidative stress in its pathogenesis.

Katre P et al studied vitamin B 12 and folic acid supplementation and plasma total homocysteine concentrations in pregnant Indian women with low B12 and high folate status and concluded that homocysteine levels were increased in non supplemented group and the levels were the same in women who received only folic acid supplementation. But pregnant women who received >1000 micrograms of vitamin B 12 had low levels of serum homocysteine[9]. This study conveys the importance of supplementation of vitamin B12 in pregnant women with adequate folic acid.

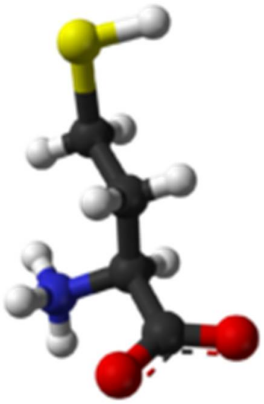
Noopur Jaiswal et al studied correlation of serum homocysteine levels and pregnancy outcome and concluded that levels of serum

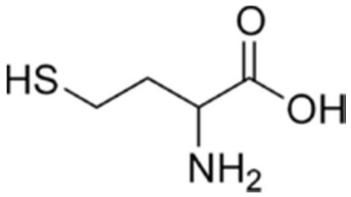
homocysteine were increased in pregnancy with complications when compared to pregnancy without complications but it was statistically not significant[{10}]. Hence this study shows that serum homocysteine levels associated with complications of pregnancy and adverse outcome is an ongoing trial and whether a particular cut off value of serum homocysteine could pre judge the complications of pregnancy is still not clear.

THEORETICAL BACKGROUND

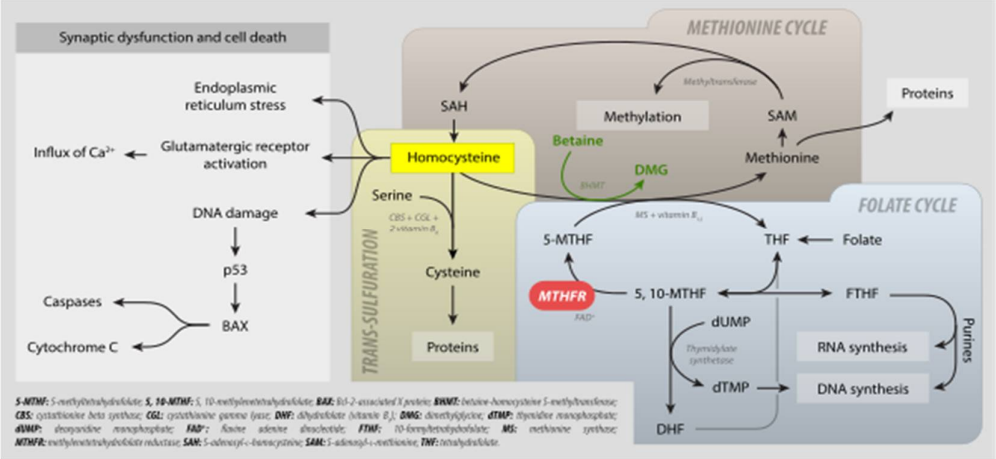
HOMOCYSTEINE:

Homocysteine is a sulphur containing amino acid needed for the growth of cells and tissues in the body. It is homologue of cysteine but differs by an added methylene bridge.





It is synthesized from methionine which receives from ATP an adenosine group and this reaction is catalyzed by S-Adenosyl methionine synthetase and the product is S-Adenosyl methionine. SAM transfers methyl group to nor epinephrine / DNA methyl transferase acceptor molecule. Then there occurs hydrolysation of adenosine to form L-homocysteine. Homocysteine so formed is converted back to L-Methionine via tetrahydrofolate or maybe converted to L-cysteine[{11}].



SOURCES OF HOMOCYSTEINE:

Homocysteine is not obtained from diet but from methionine which is found in

- Dairy products like milk, cheese and yoghurt.
- Fish and meat.
- Egg.
- Vegetables like onions, garlic, broccoli, spinach.
- Nuts and seeds like sunflower seeds, cashews, sesame seeds and pistachio.

Homocysteine levels are regulated by

- Genetic factor
- Age
- Nutritional factors
- Pregnancy

The levels of homocysteine are lower in women than in men and increases with age[12,13]. This is due to oestrogen lowering effect of homocysteine levels[14] in women and increase in homocysteine levels with increasing age is due to decreased kidney function[15,16].

Homocysteine levels are lower in children more than 1 year of age when compared to infants because folate plays an important role in children more than one year whereas Vitamin B12 plays a major role as primary modulator in infants[17,18]. From 10 years of age, boys have elevated homocysteine levels when compared to girls[19].

SERUM HOMOCYSTEINE LEVELS[20-24]

Non pregnant adult	4.4 to 10.8 micromoles/litre
First trimester	3.34 to 11 micromoles/litre
Second trimester	2.0 to 26.9 micromoles/litre
Third trimester	3.2 to 21.4 micromoles/litre

HOMOCYSTEINE CONCENTRATIONS IN PLASMA

Normal	< 10 – 12 micromoles/litre
Moderate	12 – 30 micromoles/litre
Intermediate	> 30 – 100 micromoles/litre
Severe	>100 micromoles/litre

ETIOLOGY OF HYPERHOMOCYSTEINEMIA[{25}] :

- Vitamin deficiencies :

  Folate

  Vitamin B 6 (Pyridoxine)

  Vitamin B 12 (methylcobalamine)

- Enzyme defects :

  Methylene tetrahydro folate reductase

  2 types – C 677 t

  Thermolabile variant

  Cystathionine beta synthase

  Methionine synthase

  Homozygosity is important

- Drugs

ACQUIRED FACTORS THAT INFLUENCE HOMOCYSTEINE METABOLISM

FACTOR	HOMOCYSTEINE
Folate deficiency	↑↑
Vitamin B12 deficiency	↑
Vitamin B6 deficiency	↑
Nitrous oxide	↑
Fibrate	↑
Niacin	↑
Cholestipal colestyramine	↑
Methotrexate	↑
Trimethoprim	↑
Post menopausal hormone replacement	↓
Oral contraceptives	(↑)?
Antiepileptics	↑
Metformin	↑
Omeprazole	↑
L-DOPA	↑
D-penicillamine	↓

N-acetylcysteine	↓
Tamoxifen	↓
Raloxifene	↓
Aminoglutethimide	↑
Cyclosporine-A	↑
Sulfasalazine	↑
Isoniazid	↑
Psoriasis	↑
Acute lymphocytic leukemia	↑
Rheumatoid arthritis	↑
Kidney function disorder	↑↑
Hypothyroidism	↑
Hyperthyroidism	↓
Age	↑
Post menopause	↑
pregnancy	↓
Smoking	↑
alcohol	↑

PREVALENCE :

Exact prevalence not known.

5-7 % for mild disease

MTHFR mutation

15 % in European population

1.4 % in African population

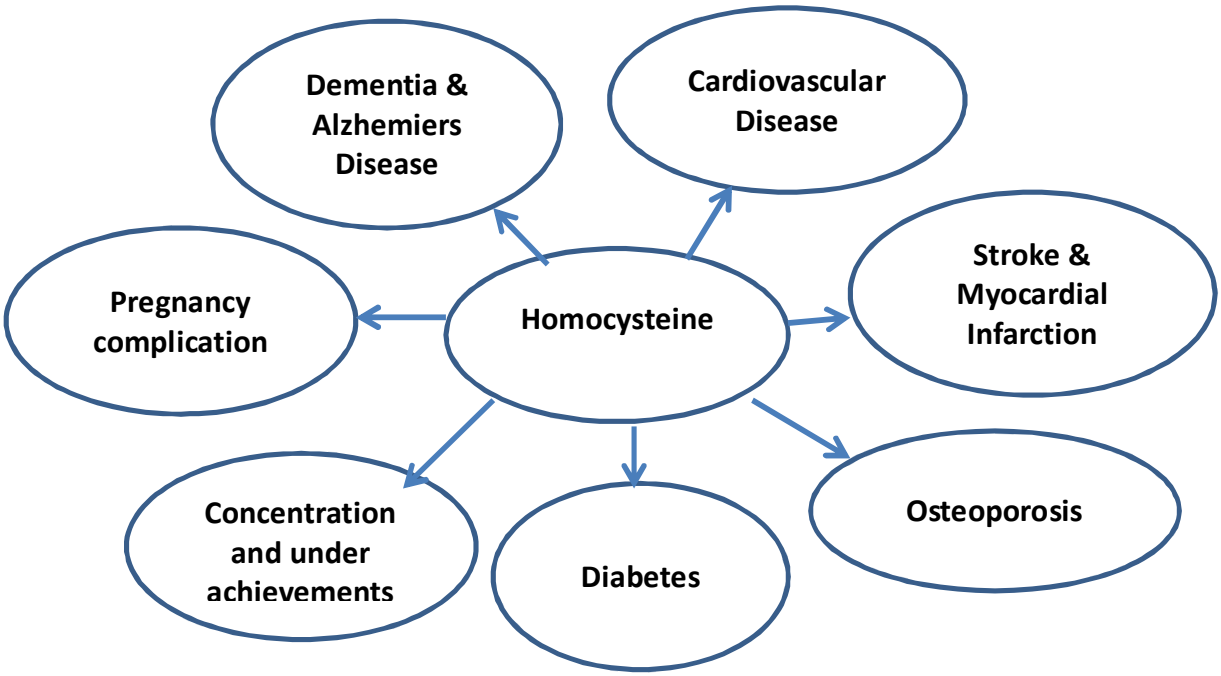
30% Heterozygous

CBS mutation

0.4 – 1.4 % Heterozygous

Rarely Homozygous

HOMOCYSTEINE & DISEASES



HOMOCYSTEINE EFFECT ON CARDIOVASCULAR SYSTEM :

- Arteriosclerotic disease
- Atherosclerosis by increasing smooth muscle size in blood vessels thereby narrowing the vessel lumen and decreasing the blood supply through reactive oxygen species[26-29].
- Deposition of ox LDL and cholesterol in the blood vessels leading to plaque formation and rupture of plaque[30].
- Increases clotting mechanism due to its reaction with apolipoprotein and plasma modified fibrin so formed leads to thrombosis[30,31-34].

HOMOCYSTEINE EFFECT ON DIABETES :

- Microangiopathy
- Macroangiopathy
- Oxidative stress leading to atherothrombotic effect.

The prognosis is worse for diabetics with homocysteine levels >14micromoles/litre[34-37].

Diabetic retinopathy prevalence is twice high in patients with homocysteine levels > 16 micromoles/litre[37].

Microalbuminuria , an indicator of glomerular damage exponentially increases with increasing levels of homocysteine.

HOMOCYSTEINE EFFECT ON DEMENTIA, ALZHEIMER'S AND COGNITIVE DYSFUNCTION :

- Increased homocysteine levels leads to decreased memory and lowers satisfaction in life[38].
- Elevated homocysteine levels in elderly people is associated with hippocampal atrophy and atrophy of cortical cerebral regions leading to increase in risk of Alzheimer's[39].
- The risk of Alzheimer's was increased to 4.5 times in patients with levels of homocysteine ≥ 14 micromoles/litre[40].
- Nilsson et al showed a link between late onset Alzheimer's and homocysteine[41].

HOMOCYSTEINE LEVELS AND CHRONIC KIDNEY DISEASE:

- Homocysteine levels are increased in renal patients,the levels being 20-80 micromoles/litre and this depends on the extent of damage to the kidneys[42,43].
- Increased levels of homocysteine is due to defect in remethylation and transsulphuration[44] and also combined with decreased intracellular levels of B12[45].
- Vitamin B and N – acetyl cysteine[46] when given in therapeutic dose through intravenous route, decreases or normalizes the levels of plasma homocysteine in patients on dialysis.
- Few longitudinal studies in patients with renal disease have showed positive association with increased homocysteine levels and mortality rate / increased coronary complications[47].
- Other studies showed better prognosis in patients with renal disease with hyperhomocysteinemia[48], termed "the reverse epidemiology" which denotes homocysteine as a marker of nutritional status.

HOMOCYSTEINE LEVELS AND OSTEOPOROSIS:

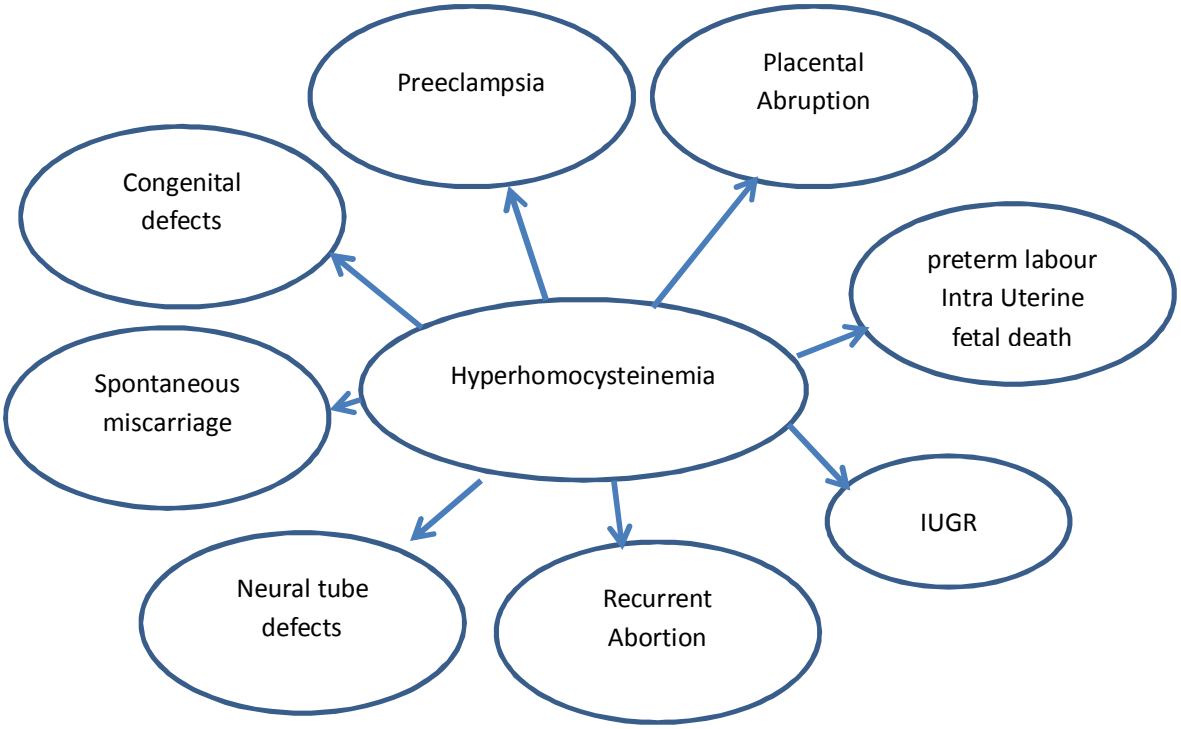
- Recent studies have shown a strong link between increased risk of osteoporotic fracture in older people and moderate elevation of homocysteine levels[49,50].

- Goerss et al showed that patients with deficiency of Vitamin B 12 leading to pernicious anaemia , have 1.9 fold increased risk of fractures of proximal femur, 1.8 fold increased risk of fractures of vertebra and 2.8 fold increased risk of fractures of distal forearm compared to controls[51], thereby showing decreased Vitamin B levels and hyperhomocysteinemia to increased risk of fractures.

HOMOCYSTEINE LEVELS AND COMPLICATIONS OF PREGNANCY:

Normally maternal levels of serum homocysteine decreases as gestational age increases. It may be due to physiological pregnancy response or maybe due to decreased albumin, increased estrogen, hemodilution occurring as a result of increase in plasma volume and increased methionine demand by mother and foetus. Another proposed mechanism is fetal utilization.

Increase in homocysteine levels in pregnant women is associated with pre eclampsia , eclampsia , abruption, HELLP syndrome, deep vein thrombosis, IUGR, prematurity,LBW,neural tube defects and recurrent miscarriages[52].



TARGET POPULATION IN WHOM SCREENING FOR HOMOCYSTEINE IS STRONGLY RECOMMENDED[25]

Patients with manifested vascular Diseases	Coronary heart disease, myocardial infarction, atherosclerosis of the carotid artery, peripheral arterial occlusive disease, atherosclerosis of the cerebral arteries, cerebral insult, venous thrombosis, pulmonary arterial embolism
People at high risk for developing vascular disease	Family history, arterial hypertension, smokers, hyperlipidaemia, renal insufficiency, diabetes, metabolic syndrome, adiposity, factor V-leiden mutation
People at risk for B-vitamins deficiency	Older people (> 65 yrs), vegetarians, inflammatory bowel diseases, gastritis, malabsorption, tropical sprue, chronic diarrhoea, renal diseases, alcohol abuse, medications
People at risk for secondary HHCY because of their clinical situation	Hypothyroidism, rheumatism, AIDS, complications during pregnancy (pre-eclampsia, HELLP syndrome), dementia, cognitive decline, Alzheimer disease, Parkinson, depression, schizophrenia, osteoporosis

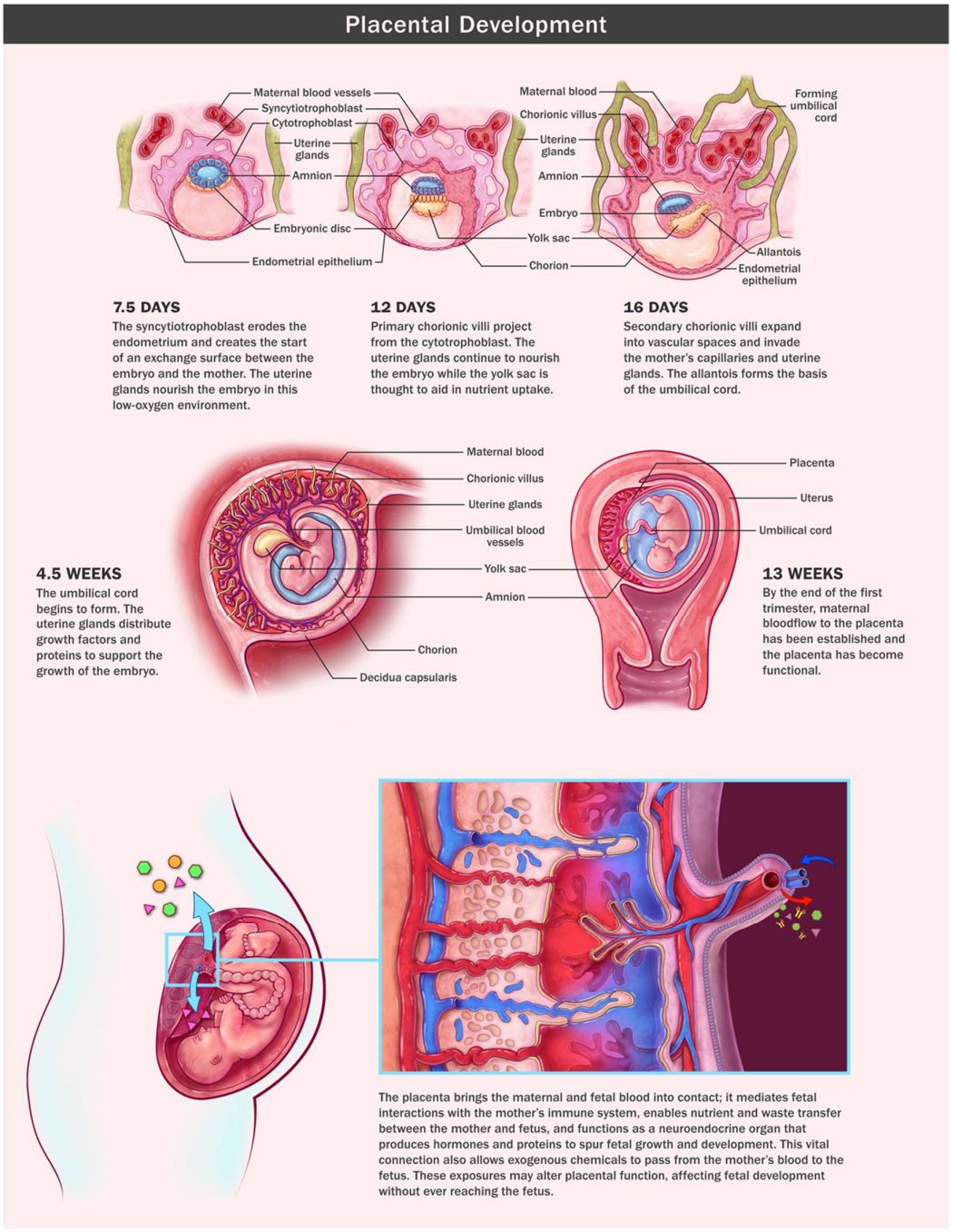
SAMPLE COLLECTION:

- Overnight fasting
- Morning sample
- EDTA tube
- Centrifuged immediately or maybe kept on wet ice till centrifuged.

METHODS:

- Chromatography
- Enzyme Immunoassay (used routinely)

DEVELOPMENT OF PLACENTA



Placenta develops from two sources. Fetal source is the chorion frondosum which forms the principal component and maternal source is the decidua basalis.

On the eleventh day after fertilization, interstitial implantation is completed and blastocyst is completely surrounded by lacunar spaces called trabeculae on all sides. Trophoblast is surrounded by outer syncytiotrophoblast and inner cytotrophoblast. Trophoblast further gives rise to villous trophoblast and extavillous trophoblast. Stem villi develops from trabeculae which later gives rise to primary , secondary and tertiary villi. Arterio – capillary- venous system is completed in the mesenchymal core of each villus on 21 st day.

The lacunar spaces filled by maternal blood and lined by syncytial cells later becomes intervillous space.

Decidua capsularis in the abembryonic area lose their villi and lacunar spaces and is converted to chorion laeve.

There occurs enormous subdivision of the villi in the embryonic pole and extensive proliferation of decidua basalis forming discrete placenta which is completed by 12 th week.

Hemochorial development of placenta needs endometrial and spiral arterial invasion[{53}].This occurs with the help of regulatory factors under conditions of hypoxia[{54}].

MMP – 9 plays an important role in trophoblast invasion and this in turn is regulated by insulin like growth factor 2 secreted by trophoblast which promotes endometrial invasion and this is blocked by insulin like growth factor binding protein type 4 secreted by decidual cells.

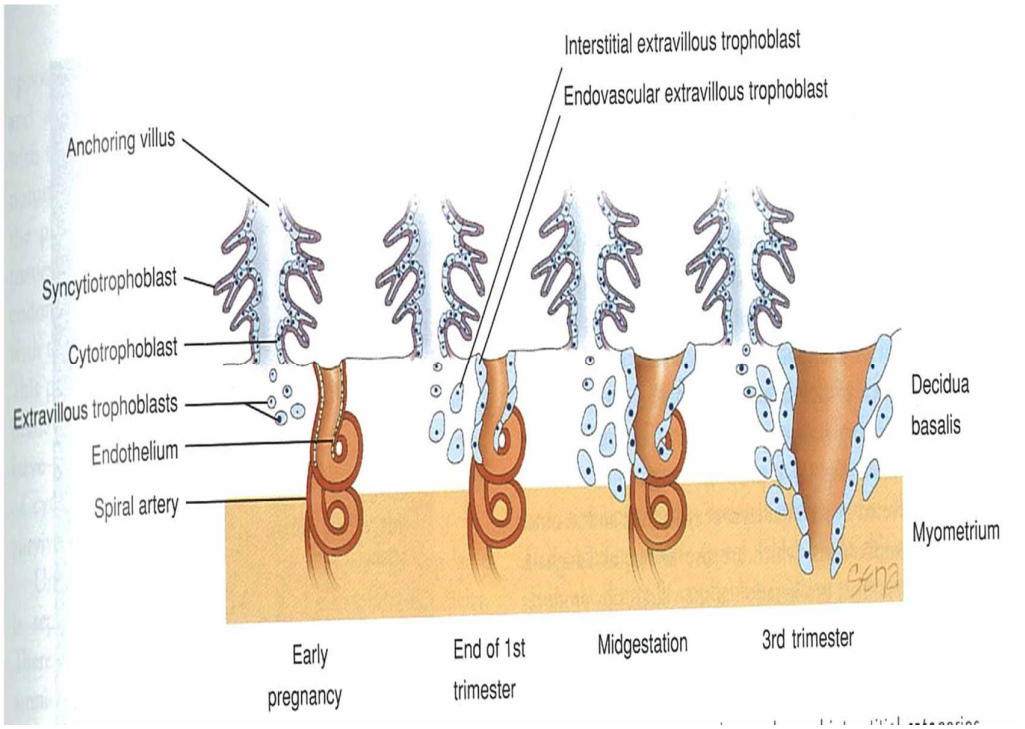
Low levels of estrogen in first trimester of pregnancy is essential for remodelling of spiral arteries and invasion of trophoblast.

Extra villous trophoblast forms interstitial trophoblast and endovascular trophoblast.

The interstitial trophoblast clumps together around spiral arteries and also penetrates into the decidua and myometrium and prepares the spiral vessels for endovascular invasion of trophoblast[{55}].

Endovascular trophoblasts enter the spiral arteries and forms cellular plugs, then by apoptosis damages the vascular endothelium and alters the vascular media and causes fibrinoid deposition. Spiral artery then regenerates the endothelium. This trophoblastic invasion involves only the spiral arteries in decidua basalis and does not involve the decidual veins[{56}].

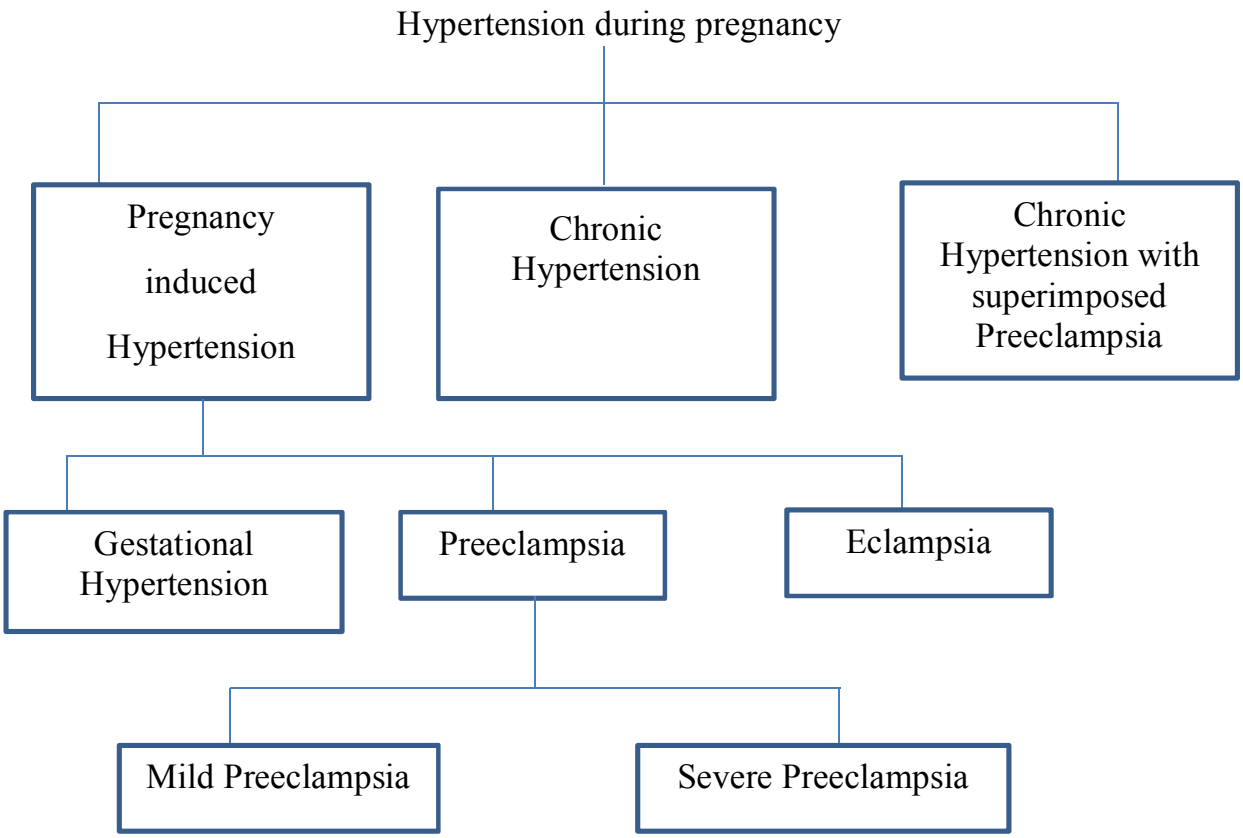
Thus , this development occurs in two stages.The first stage occurs post fertilization upto 12 weeks and involves remodelling of spiral arteries upto decidual myometrial border. The second stage occurs in 12 to 16 weeks , in which there occurs some invasion of spiral arteries in the intramyometrium , thereby converting the muscular , small lumen spiral arteries into low resistance, high flow and high volume uteroplacental circulation[53].



CLASSIFICATION OF PREGNANCY INDUCED HYPERTENSION

National High Blood Pressure Education Programme – 2000

Updated by ACOG 2013



DIAGNOSTIC CRITERIA FOR PREGNANCY ASSOCIATED HYPERTENSION(ACOG 2013)

Gestational Hypertension

Blood pressure > 140 / 90 mm Hg in previously normotensive women after 20 weeks of gestation.

Pre eclampsia

Hypertension and

Proteinuria > or = 300 mg / 24 hours

Protein creatinine ratio > or = 0.3

Dipstick 1 + persistant

Or

Thrombocytopenia platelets < 1 lakh / microlitre

Renal insufficiency creatinine > 1.1 mg / dl or doubling of baseline.

Liver involvement serum transaminase levels twice Normal.

Cerebral symptoms Headache ,visual disturbances , Convulsions.

Pulmonary edema

Pre eclampsia :

It is a pregnancy specific syndrome of unknown etiology and is characterized by development of hypertension > or = 140/90 mm Hg with proteinuria after 20 weeks of gestation involving multiple systems in previous normotensive and non proteinuric women.

Eclampsia :

Convulsions in women who also meets the criteria for pre eclampsia.

Associated neurological disease ruled out.

Chronic Hypertension :

Hypertension developing < 20 weeks of gestational age .

Hypertension persistent beyond 12 weeks postpartum.

Superimposed pre eclampsia :

Development of pre eclampsia in women with chronic hypertension

INDICATORS OF SEVERITY OF HYPERTENSIVE DISORDERS[57] :

	NON SEVERE	SEVERE
Systolic BP	< 160 mm Hg	>or = 160 mm Hg
Diastolic BP	< 110 MM Hg	>or = 110 mm Hg
Proteinuria	None to positive	>5 g in 24 hours
Oliguria	Absent	<400 ml / 24 hours
Headache	Absent	Present
Visual disturbances	Absent	Present
Persistent epigastric pain	Absent	Present
Thrombocytopenia	Absent	Present < 1 lakh
Serum transaminase levels	Minimal elevation	Marked elevation
Serum creatinine	Normal	Elevated
Pulmonary edema	Absent	Present
IUGR	Absent	Present
Convulsion	Absent	Present

INCIDENCE :

Incidence in nullipara is 3-10 %[{58}]

Genetic predisposition , race , ethnicity, socio economic and seasonal changes influence the incidence of pregnancy induced hypertension[{59}].

Incidence of pre eclampsia in singleton pregnancy – 5 %.

Incidence of pre eclampsia in twin pregnancy – 13 %.[{60}]

RISK FACTORS :

- Primi gravida
- Multifetal gestation
- Molar pregnancy
- Diabetes complicating pregnancy
- Obesity
- Family history of hypertension
- Pre existing vascular disease
- New paternity
- Thrombophilias
- Women with pre eclampsia in previous pregnancy are at greater risk for developing pre eclampsia in second pregnancy[{62}].

Kraus et al mentioned that smoking upregulates placental adrenomedullin expression which upregulates volume homeostasis and decreases risk of hypertension during pregnancy[{61}]

ETIOPATHOGENESIS :

- Exposed to chorionic villi for the first time
- Inflammation or endothelial cell activation
- Genetic predisposition
- Exposure to abundant chorionic villi as in molar pregnancy and multifetal gestation.

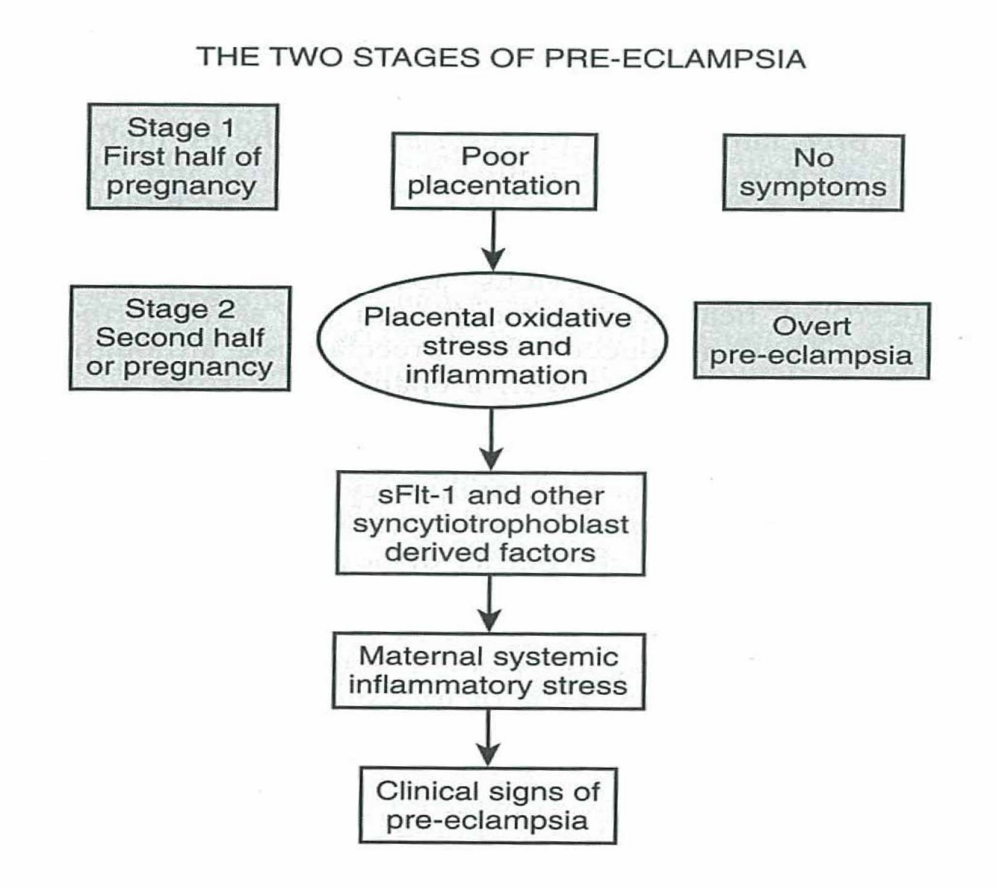
"2 stage disorder" theory as proposed by N**ESS AND ROBERTS** includes "maternal and placental pre eclampsia".

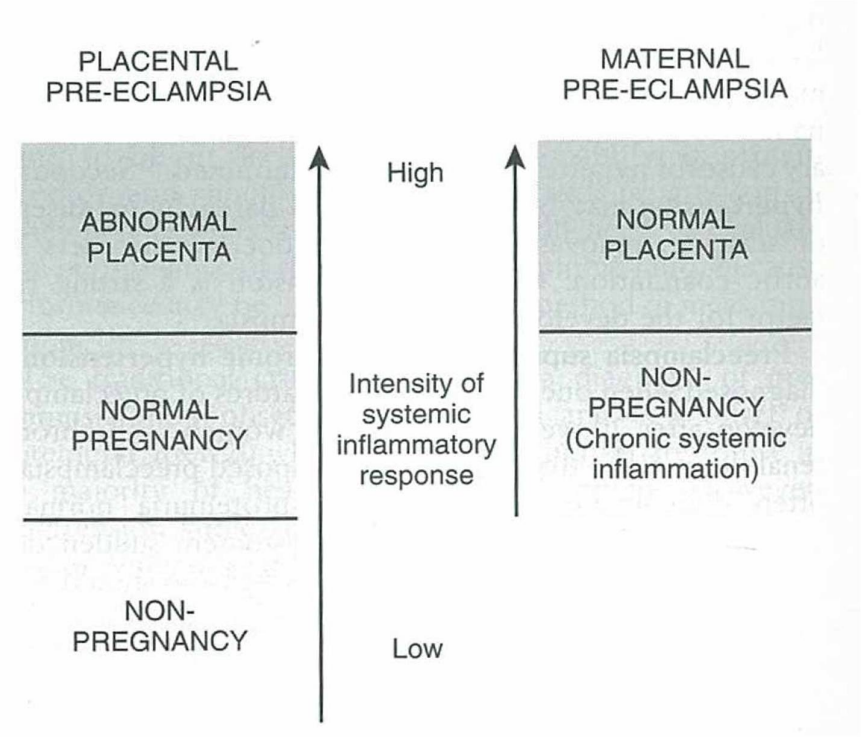
REDMAN & CO WORKERS proposed that

Stage 1 Faulty remodelling of endovascular trophoblasts.

Stage 2 Clinical syndrome.

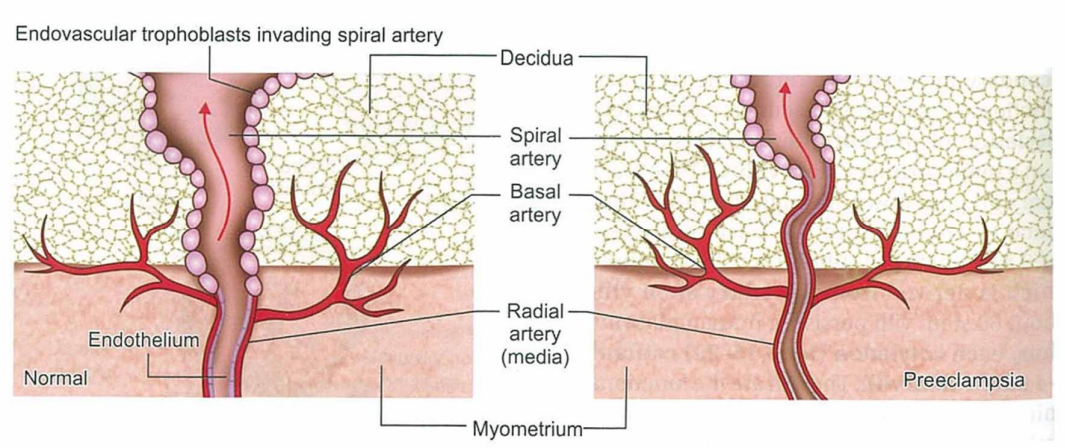
Stage 2 is modified by maternal conditions by endothelial cell activation or inflammation.


THE TWO STAGES OF PRE-ECLAMPSIA
Stage 1
First half of pregnancy
Poor placentation
No symptoms
Stage 2
Second half or pregnancy
Placental oxidative stress and inflammation
Overt pre-eclampsia
sFlt-1 and other syncytiotrophoblast derived factors
Maternal systemic inflammatory stress
Clinical signs of pre-eclampsia


PLACENTAL PRE-ECLAMPSIA
MATERNAL PRE-ECLAMPSIA
ABNORMAL PLACENTA
NORMAL PREGNANCY
NON-PREGNANCY
High
Intensity of systemic inflammatory response
Low
NORMAL PLACENTA
NON-PREGNANCY (Chronic systemic inflammation)

ETIOLOGY :

- ABNORMAL TROPHOBLAST INVASION



Trophoblastic invasion is incomplete in which endovascular trophoblast line the decidual arteries but not the myometrial arterioles[{63}].

Decreased soluble anti angiogenic growth factors. Endothelial cell damage ,myointimal cell proliferation and medial necrosis.

Vascular lesions leading to narrowing of spiral arterioles ,atherosis and infarcts.

- IMMUNOLOGICAL FACTORS :

MHC antigens are absent in villous trophoblasts but MHC Class 1 is expressed on invasive extra villous trophoblasts.

HLA G plays an important role by preventing immune rejection of extravillous trophoblasts by modulating the functions of uterine natural killer cells[64].

There occurs abnormal HLA G expression in pre eclampsia in extra villous trophoblasts[65].

Dysregulation of maternal immunological tolerance to fetal antigens and paternal derived placental antigens is another proposed mechanism in pre eclampsia[66].

- ENDOTHELIAL CELL ACTIVATION

Endothelial injury caused by TNF- alpha and interleukins may lead to oxidative stress due to free radicals leading to self propagating formation of lipid peroxides[67] thereby modifying production of nitric oxide and causing prostaglandin imbalance.

- NUTRITIONAL FACTORS:

  - Diet rich in fruits and vegetables having antioxidant activity lowers blood pressure.
  - Trials showed that antioxidant vitamins like vitamin C or E supplementation did not show any added benefits.

- GENETIC FACTORS:

GENE	FUNCTION AFFECTED
MTHFR(C6771)	Methylene tetrahydrofolate reductase
F5(Leiden)	Factor V
AGT(M235T)	Angiotensinogen
HLA	Human leukocyte antigen
NOS3(Glu 298 Asp)	Endothelial nitric oxide
F2(G20210A)	Prothrombin
ACE	Angiotensin converting enzyme
CTLA4	Cytotoxic T- lymphocyte associated protein
SERPINE 1	Serine peptidase inhibitor

FUNCTIONS OF NORMAL ENDOTHELIUM :

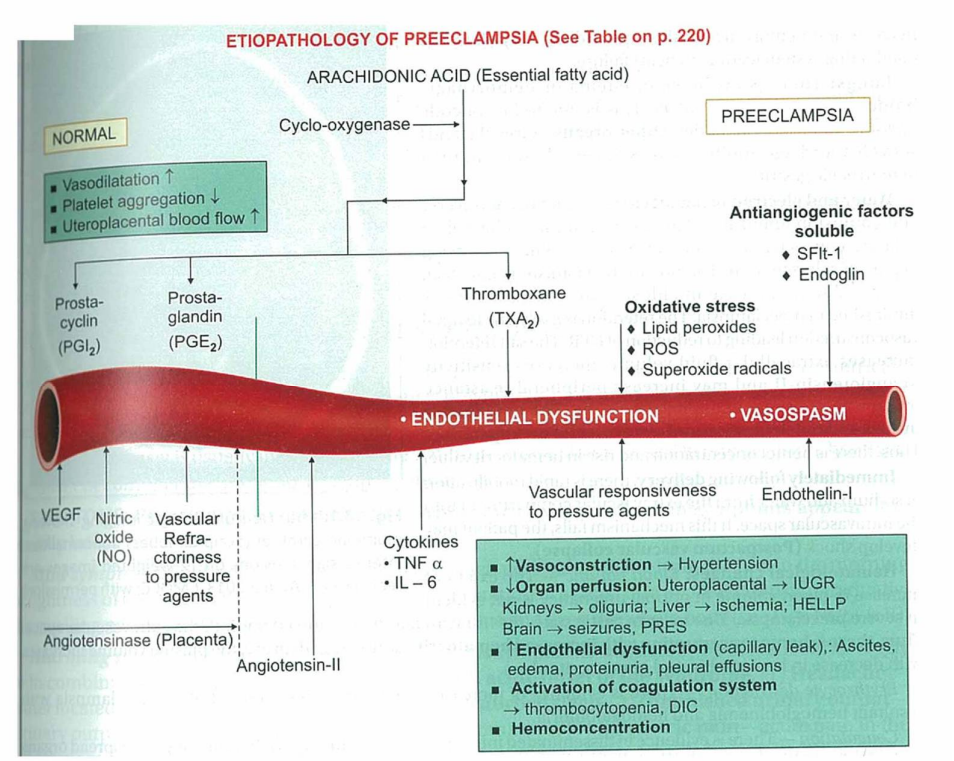
Two major functions of intact endothelium

-coagulation prevention

-vascular tone modulation

Central role in PIH is endothelial injury.

Features of PIH are preceeded by endothelial dysfunction.



PATHOGENESIS:

➢ VASOSPASM

- vasoconstriction as a result of endothelial activation.
- Damage to endothelial cells leading to interstitial leakage of platelets and fibrinogen.
- subendothelial deposition leading to diminished blood flow.
- intense attenuation of blood volume in severe pre eclampsia[{68}].

- ENDOTHELIAL CELL INJURY

- circulating endothelial cell levels in peripheral blood is increased[{69}].
- increased sensitivity to vasopressors.
- endothelial cell damage promotes substances leading to coagulation[{70}].
- increased endothelial microparticles in circulation[{71}].
- decrease in prostacyclin and thromboxane A2 ratio.
- increase in nitric oxide inactivation due to decreased expression of endothelial nitric oxide synthase[{72}].
- increase in endothelin 1 which is a potent vasoconstrictor.

PATHOPHYSIOLOGY

- **CARDIOVASCULAR SYSTEM**

- increase in cardiac after load due to hypertension.
- decrease in hypervolemia of pregnancy leading to decreased cardiac preload.
- endothelial activation with extravasation to the extracellular spaces.
- increase in peripheral vascular resistance leads to decrease in cardiac output[{73}].

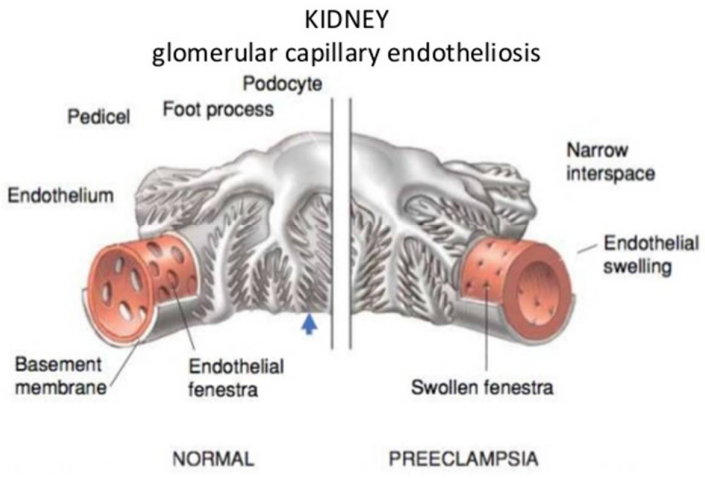
- **HEMATOLOGICAL CHANGES:**

- decrease in blood volume expansion to the extent of 16% which is about 50% in normal pregnant women[{74}].
- marked hemo concentration
- endothelial injury leading to microangiopathic hemolysis.
- Thrombocytopenia
- increase in fibronectin

- **HORMONAL CHANGES**

- increased sensitivity to vascular pressor agents like angiotensin 2, cathecolamines and vasopressin leading to increase in vascular resistance.
- alteration in prostacyclin and thromboxane A2 ratio.
- Increase in levels of pro atrial natriuretic peptide.

- **KIDNEY**



- renal blood flow and glomerular filtration rate are increased in normal pregnancy.
- decrease in renal perfusion and glomerular filtration occurs in pre eclampsia.
- decrease in glomerular filtration rate is due to increased resistance of renal afferent arterioles.

Microscopic Features

- glomerular capillary endotheliosis.
- deposition of proteins and fibrin like material subendothelially.

Increase in sr. creatinine levels due to decreased GFR.

Increase in plasma uric acid is due to increased tubular reabsorption.

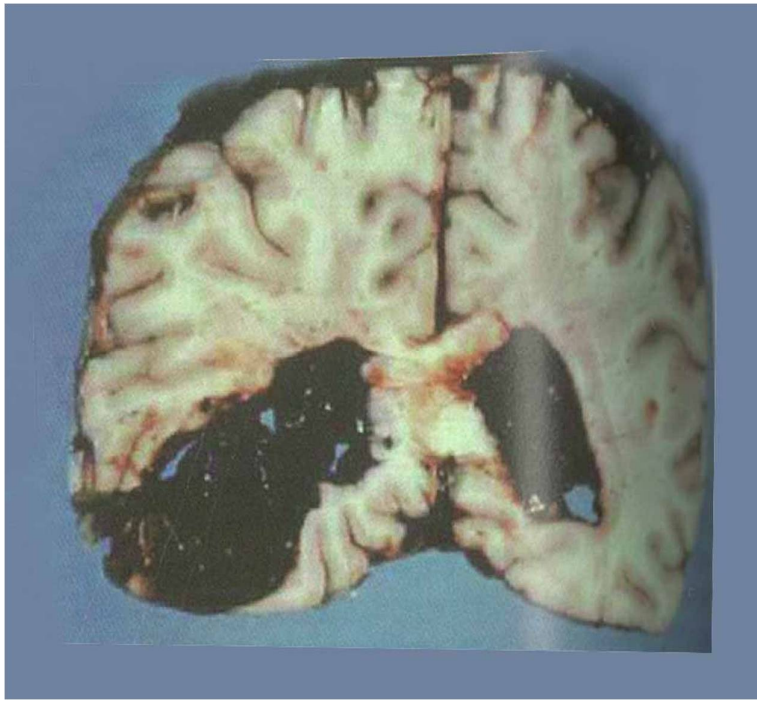
- **LIVER**

- Characteristic feature is periportal haemorrhage in the periphery of liver.
- Haematoma in subcapsular region.
- haemorrhage and rupture of glisson's capsule leading to life threatening intraperitoneal bleed.
- elevation in levels of serum hepatic transaminases.

- In HELLP syndrome there occurs, hemolysis, elevated liver enzymes and low platelet counts.

➢ **BRAIN**

- cerebral blood flow and cerebral oxygen metabolism is normal in pre eclampsia[{75}].
- Increase in cerebral vascular resistance occurs in pre eclampsia.
- Subcortical edema, softening of brain and haemorrhage in white matter occurs.
- Parieto occipital cortex is most frequently affected.
- cerebro vascular hyperperfusion manifests as headache and scotomata.
- Eclampsia occurs due to release of glutamate excessively which is a excitatory neurotransmitter, bursts of action potential and massive depolarization of neurons.
- On MR imaging- hyperintense T2 leisons in subcortical and cortical areas of parieto occipital lobes.
- Basal ganglia, brainstem and cerebellum are also involved.



- **RETINA**

GRADING OF HYPERTENSIVE RETINOPATHY

GRADE 1: Slight to moderate narrowing of retinal vessels with A: V ratio>1:2

GRADE 2: Moderate to severe narrowing of retinal arteries with arterio: Venous ratio<1:2

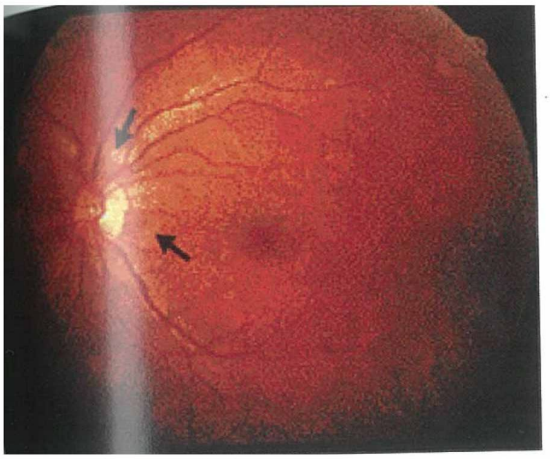
GRADE 3: Soft exudates and flame shaped haemorrhages.

GRADE 4: Grade 3 changes and bilateral optic nerve edema.

Blindness is rare and is reversible and it is due to

- occipital lobe involvement – amaurosis,

- Retinal involvement – serous retinal detachment / infection called as purtscher retinopathy.



➢ **EFFECT ON FETUS**

- uteroplacental insufficiency
- intrauterine growth restriction
- Oligohydraminos.
- Preterm labour
- increased risk of abruption.

PREDICTIVE TESTS FOR PRE ECLAMPSIA

➢ **Placental Perfusion/Vascular Resistance**

- Roll over test
- Isometric handgrip test

- Pressor response to aerobic exercise
- Mid trimester mean arterial pressure
- Angiotensin II infusion
- Uterine artery or fetal transcranial Doppler velocimetry

➢ **Fetal Placental Unit**

- Human chorionic gonadotrophin
- Alpha feto protein
- Pregnancy associated protein A
- Estriol
- Inhibin A
- Activin A
- Placental protein 13
- CRH
- ADAM Metallopeptidase domain 12
- Kisspeptin

- **Renal Dysfunction**
  - Serum uric acid
  - Micro Albuminuria
  - Urinary calcium/kallikrein
  - N Acetyl beta glucosaminidase
  - Micro transferrinuria
  - Cystatin C
  - Podocyturia
- **Endothelial Dysfunction**
  - Platelet count and activation
  - Fibronectin
  - Endothelial adhesion molecules
  - C reactive protein
  - Homocysteine
  - Angiogenic factors
  - Fms – like tyrosine kinase receptor -1
  - Endoglin

- **Others**
    - Antithrombin III
    - Atrial natriuretic peptide
    - Beta II microglobulin
    - Genetic markers
    - Cell free fetal DNA
    - Serum and urine proteomics
    - Metabolomic markers
    - 25 hydroxy vitamin D

HYPERHOMOCYSTEINEMIA IN PRE ECLAMPSIA

- Causes endothelial damage.
- Thromboembolic effects
- Placental hypoperfusion.
- Excess production of toxic free radicals

ECLAMPSIA

Preeclampsia complicated with generalized tonic-clonic convulsions /coma.

seizures ,most commonly occurs in third trimester.

Seizures can occur

- ANTE PARTUM

  It constitutes upto 50% of cases.

  Seizures occur most often before start of labour and labour ensues soon after.

- INTRAPARTUM

  It constitutes upto 30% of cases.

- POSTPARTUM

  Contributes upto 20%

  seizures occurring for first time within 48-72 hours after delivery.

- LATE POSTPARTUM

  Seizures occurring beyond 48 hours of child birth but less than 4 weeks.

STAGES

- PREMONITORY STAGE

- lasts for 30 seconds.

- patient becomes unconscious.
- muscles of face, tongue and limbs undergo twitching
- Eyeballs roll and become fixed.

➢ TONIC STAGE

- Lasts for 30 seconds.
- tonic spasm of the body occurs
- tongue protrudes
- Respiration stops.
- cyanosis appears
- eyeballs fixed

➢ CLONIC STAGE

- Lasts for 1-4 minutes.
- Alternate contraction and relaxation
- Breathing becomes stertorous.
- cyanosis disappears.

➢ STAGE OF COMA

CAUSE

- Anoxia
- cerebral edema
- cerebral dysrhythmia

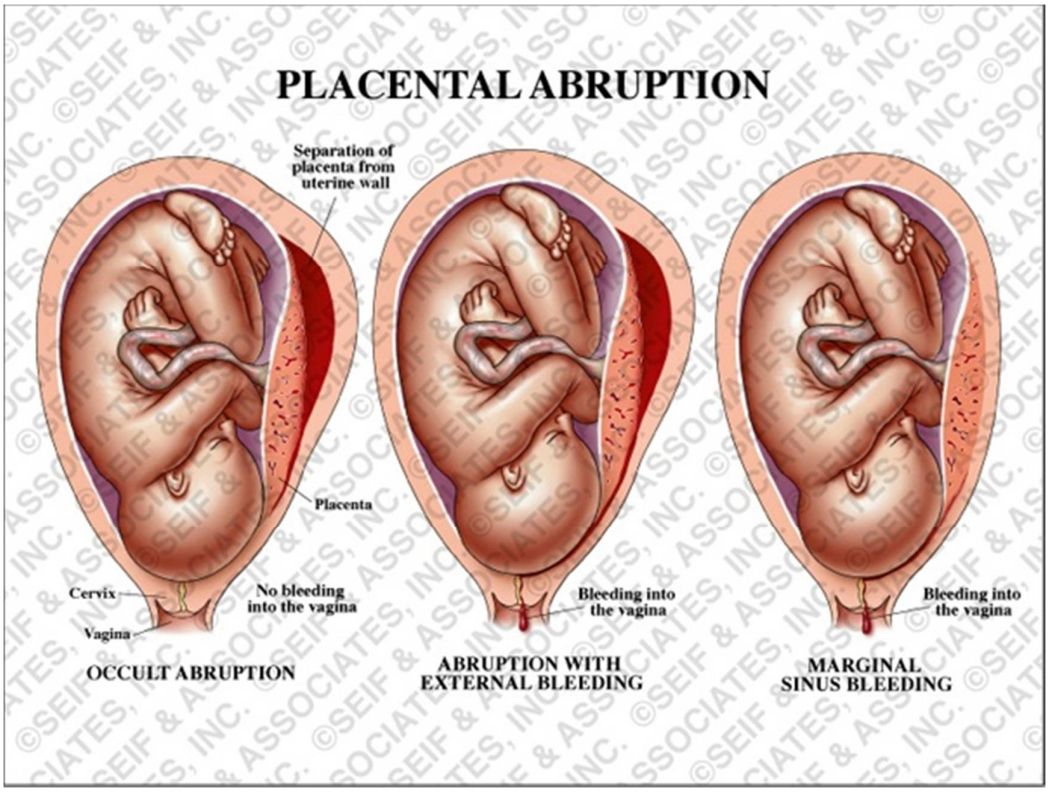
ABRUPTIO PLACENTA

It is the premature separation of normally situated placenta.

VARIETIES

- Revealed
- Concealed
- Mixed

Bleeding is mostly maternal rarely fetal as in traumatic abruption .



INCIDENCE:

Occurs in 1 in 200 deliveries.

Perinatal mortality 15-20%

Maternal mortality 2-5%

ETIOLOGY

- DEMOGRAPHIC FACTORS

- Higher birth order:

  3 times more common in Gravida 5 and above (Pritchard 1991) Torhey 1993

- Advancing maternal age

  Women > 40 years have 2-3 times increased risk of abruption when compared to women ≤35years.(Cleary Goldman ,2005)

- Race or ethinicity

- Familial association

  Women having severe abruption , the risk of abruption was doubled in her sister and heritability risk was 16%[80]. (Rasmussen, 2009).

- HYPERTENSION AND PRE ECLAMPSIA

  Zetterstrom and associates found increased incidence of abruption in chronic hypertensives compared to normotensive women[{78,79}]. Pre eclampsia superimposed on chronic hypertension confers greater risk of abruption.

- TRAUMA

- PRETERM PREMATURE RUPTURE OF MEMBRANES

- CIGARETTE SMOKING

- COCAINE ABUSE

- THROMBOPHILIAS AND LUPUS ANTICOAGULANT

- UTERINE LEIOMYOMAS

- RECURRENT ABRUPTION

In case of previous mild abruption recurrent risk is 6.5 fold increased and with severe abruption recurrent risk is 11.5 fold increasesd.

Women with history of two severe abruptions, the risk is increased 50 fold.

PATHOGENESIS

- Impaired invasion of trophoblasts with atherosis is seen in some cases of pre eclampsia and abruption[{76}] (Brosens, 2011)
- Infection /Inflammation as proposed by Nath and Collegues[{77}].
- Bleeding into the deciduas basalis
- Decidual hematoma forms

CHANGES IN ORGANS

- COUVELAIRE UTERUS

  Also known as uteroplacental apoplexy.

  Naked eye – uterus is port wine colour which is patchy or diffuse

  Subperitoneal haemorrhages

  Necrosis of the affected area

  Infiltration of blood and fluid between muscle bundles.

- ACUTE KIDNEY INJURY
- CONSUMPTIVE COAGULOPATHY
- SHEEHAN SYNDROME

CLINICAL CLASSIFICATION DESCRIBED BY PAGE

GRADE 0 – Clinical features absent.

Diagnosis made only on placental inspection

GRADE I – Vaginal bleeding is minimal.

Uterus irritable and tenderness absent.

No Signs of maternal shock or fetal distress.

GRADE II – Vaginal bleeding is mild to moderate

Uterine tenderness present

No signs of maternal shock

Fetal distress/ fetal death present

GRADE III – Bleeding moderate to severe / concealed.

uterine tenderness marked.

Shock present

Fetal death

Coagulation disorder/ anuria may be present

CLINICAL FEATURES OF REVEALED AND MIXED VARIETY

PARAMETERS	REVEALED	MIXED
Symptoms	Abdominal pain followed by bleeding per vaginum	Intense acute abdominal pain followed by slight vaginal bleeding
Character of bleeding	Slight to moderate, continuous dark colour	Slight , continous dark colour
General condition	Proportionate to visible blood loss	Shock is pronounced
Pallor	Related to visible blood loss	Severe , out of proportion to visible blood loss
Features of Pre eclampsia	May be present	Frequent association

Uterine height	Proportionate to gestational age	May be disproportionately enlarged
Uterine feel	Normal with localized tenderness, contractions frequent	Uterus is tense , tender and rigid
Fetal parts	Can be made out easily	Not made out easily
Fetal heart sound	Usually present	Usually absent
Urine output	Normal	Usually decreased
Lab tests		
Haemoglobin	Low , proportionate to blood loss	Markedly lower, out of proportion to visible blood loss
Coagulation profile	Normal	Clotting time increased Platelet count low Fibrinogen level low Increased APTT Increased FDP and D-dimer

MATERIALS AND METHODS

Study design - prospective study

Study place - Department of obstetrics and gynecology, Coimbatore medical college hospital

Study duration - Oct 2016 to Sep 2017

Inclusion criteria:

- Booked antenatal women 21-35 years of age
- Gestational age 34-40 weeks

Exclusion Criteria

- Diabetes mellitus
- Essential Hypertension
- Recurrent pregnancy loss
- Liver disease
- Multiple pregnancies
- Severe Anaemia
- Smoking and tobacoo chewers

- Pregnancy with APLA syndrome
- Polyhydraminos
- Thyroid disorders

Group I

Sample size of 40 normotensive healthy pregnant women fullfilling the inclusion criteria.

Group II

Sample size of 40 pregnant women in inclusion criteria with BP ≥ 140/90mm Hg with proteinuria > 2+ in dipstick in random urine sample.

Group III

Sample size of 40 pregnant women satisfying the inclusion criteria with onset of generalized tonic clonic seizures /coma superimposed on pre eclampsia.

Group IV

Sample size of 40 pregnant women with abruption included in inclusion criteria with clinical and USG diagnosis.

During the period of Oct 2016 to Sep 2017, patients coming to Obstetrics and gynaecology department in Coimbatore medical college hospital with above criteria are grouped.

A detailed history including patient's age race, parity, socioeconomic status menstrual, medical history, obstetric, past, dietary and treatment history noted.

General examination, systems and obstetric examinations done. Routine investigations like blood grouping, haemoglobin, urine albumin sugar, HIV, HbsAg, Blood Sugar, VDRL and Ultra Sound are done.

Women in group II, III and IV - special investigations of blood urea, serum creatinine, platelet count, Serum lactate dehydrogenase, Bleeding time,Clotting time, liver enzymes, sr. uric acid were done. Serum homocysteine levels were done in all patients. 5ml of venous blood drawn from antecubital vein and sent in labelled bottles to lab immediately and centrifuged within 3000 rpm for 5 mins . Serum separated is analysed by chemilumniscent microparticle immuno assay and values were recorded.

OBSERVATION AND RESULTS

The following pages are the tables and graphs which gives us the descriptive analysis of 160 patients in the study according to age, parity, gestational age, prepregnancy BMI, previous history, risk factors, investigations, maternal and fetal complications, birth weight and serum homocysteine levels.

TABLE -1

DISTRIBUTION OF PATIENTS ACCORDING TO AGE

Age	Frequency	Percent
21-30 yrs	118	73.8
31-35 yrs	42	26.2
Total	160	100.0

In this study 118 (73.8%) patients belong to age group 21 to 30 years and 42 patients (26.2%) belong to age group 31 to 35 years.

TABLE-2

DISTRIBUTION OF PATIENTS ACCORDING TO PARITY

Parity	Frequency	Percent
First pregnancy	78	48.8
Second pregnancy	61	38.1
Third pregnancy or more	21	13.1
Total	160	100.0

78 Patients in this study are primigravida, 61 patients are 2nd gravida and 21 patients are 3rd gravida or more.

TABLE-3

DISTRIBUTION OF PATIENTS ACCORDING TO

GESTATIONAL AGE

Gestational Age	Frequency	Percent
34 - 36 weeks	67	41.9
37 - 40 weeks	93	58.1
Total	160	100.0

67 Patients in this study are in 34 to 36 weeks of gestation and 93 patients are in 37 to 40 weeks of gestation.

TABLE-4

DISTRIBUTION OF PATIENTS ACCORDING TO PRE PREGNANCY BMI

BMI	Frequency	Percent
>30	12	7.5
<30	148	92.5
Total	160	100.0

Increase in prepregnancy BMI has an increased risk of developing PIH, which was proved from many studies, hence this factor is included in this study. 7.5% of patients in this study have BMI more than 30 and 92.5% have BMI less than 30.

TABLE-5

DISTRIBUTION OF PATIENTS ACCORDING TO PREVIOUS HISTORY

Previous History	Frequency	Percent
Preeclampsia	14	8.8
Eclampsia	1	.6
Abruption	6	3.8
No	61	38.1
Not applicable	78	48.8
Total	160	100.0

14 patients have previous history of pre eclampsia, 1 patient has history of eclampsia and 6 have past history of abruption.

TABLE-6

DISTRIBUTION OF PATIENTS ACCORDING TO RISK FACTOR

Risk Factor	Frequency	Percent
Mild Preeclampsia	21	13.1
Severe Preeclampsia	19	11.9
Eclampsia	40	25.0
Abruption	40	25.0
No risk factor/ Normal pregnancy	40	25.0
Total	160	100.0

In this study 21 patients have mild pre eclampsia, 19 patients have severe pre eclampsia, 40 patients have eclampsia and 40 patients have abruption.

TABLE-7

DISTRIBUTION OF PATIENTS ACCORDING TO INVESTIGATIONS

Investigations	Frequency	Percent
Abnormal	85	53.1
Normal	75	46.9
Total	160	100.0

In this study investigations were abnormal in 85 patients and were normal in 75 patients.

TABLE-8

DISTRIBUTION OF PATIENTS ACCORDING TO MATERNAL COMPLICATIONS

Maternal complications	Frequency	Percent
Renal failure	11	6.9
HELLP	6	3.8
CVT	13	8.1
DIVC	2	1.2
Pulmonary complications	2	1.2
Shock	1	.6
PPH	1	.6
Sepsis	1	.6
Maternal Death	1	.6
No complications	122	76.2
Total	160	100.0

No complications occured in 122 patients. 13 patients developed CVT, 11 patients developed renal failure and maternal death occurred in one.

TABLE-9

DISTRIBUTION OF BABIES ACCORDING TO BIRTH WEIGHT

Birth Weight	Frequency	Percent
>2.5 - 3.5	82	51.2
2 - 2.5	60	37.5
<2	18	11.2
Total	160	100.0

In this study, 82 babies weighed more than 2.5 kg, 60 babies weighed between 2 to 2.5 kg and 18 babies weighed less than 2 kg.

TABLE-10

DISTRIBUTION OF BABIES ACCORDING TO FETAL COMPLICATIONS

Fetal complications	Frequency	Percent
Preterm	52	32.5
IUGR	23	14.4
Fetal death	8	5.0
No	77	48.1
Total	160	100.0

In this study 77 babies are normal, 52 babies are pre term, 23 are IUGR and fetal death occurred in 8.

TABLE 11

DISTRIBUTION OF PATIENTS ACCORDING TO AGE AND RISK FACTOR

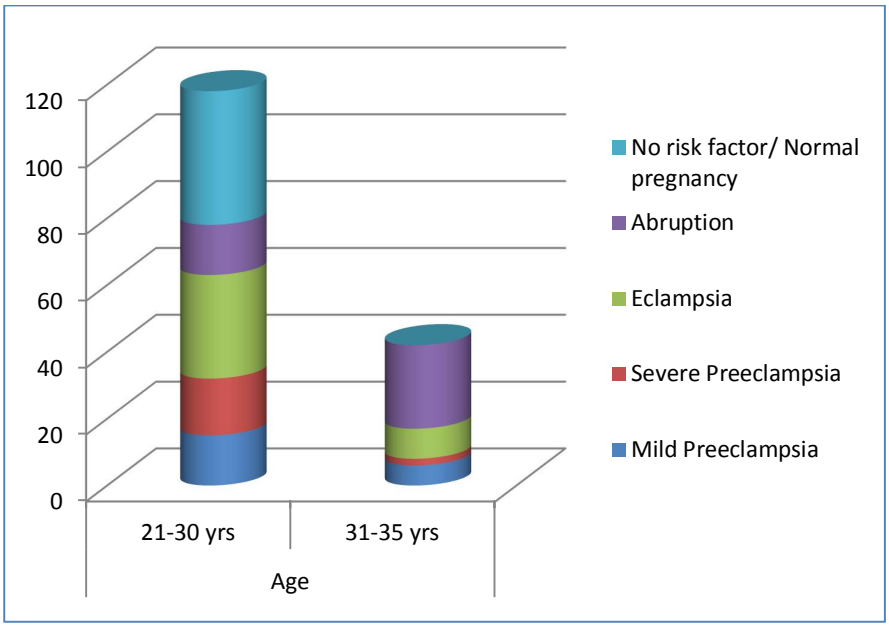
			Risk Factor					Total	P value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor/ Normal pregnancy		
Age	21-30 yrs	No	15	17	31	15	40	118	0.000**
		%	71.4%	89.5%	77.5%	37.5%	100.0%	73.8%	
	31-35 yrs	No	6	2	9	25	0	42	
		%	28.6%	10.5%	22.5%	62.5%	.0%	26.2%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	

* Significant at 5% level of significance

** Significant at 1% level of significance

GRAPH-1

DISTRIBUTION OF PATIENTS ACCORDING TO AGE AND RISK FACTOR



From the above table and graph, it is seen that mild pre eclampsia is seen in 71.4%, severe pre eclampsia in 89.5% and eclampsia is seen in 77.5% of patients in 21 to 30 years of age. Abruption is seen in 62.5% patients in 31 to 35 years of age with p value at 1% level of significance.

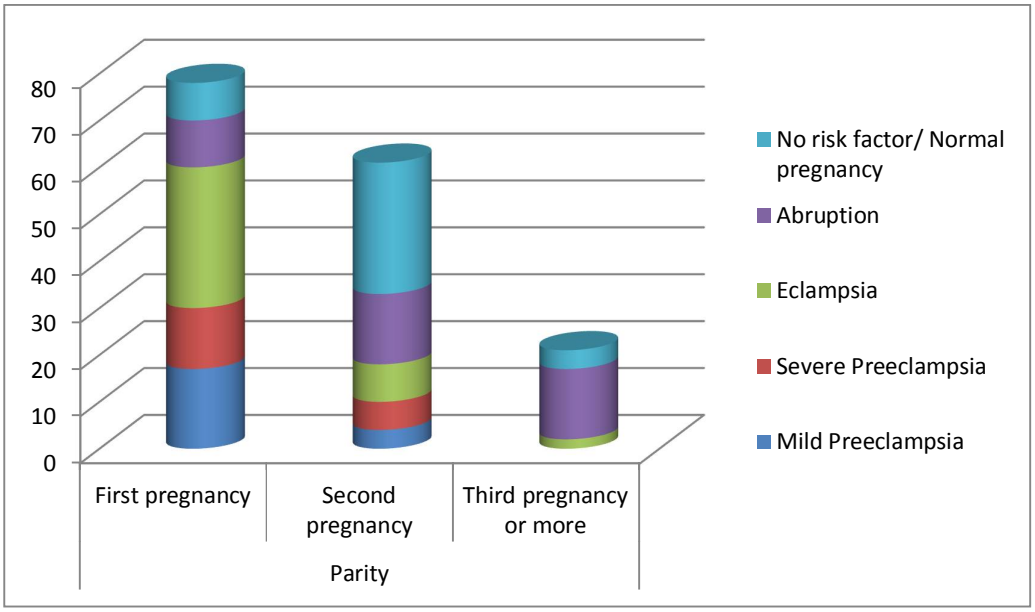
TABLE-12

DISTRIBUTION OF PATIENTS ACCORDING TO PARITY AND RISK FACTOR

			Risk Factor					Total	P value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor/ Normal pregnancy		
Parity	First pregnancy	No	17	13	30	10	8	78	0.000**
		%	81.0%	68.4%	75.0%	25.0%	20.0%	48.8%	
	Second pregnancy	No	4	6	8	15	28	61	
		%	19.0%	31.6%	20.0%	37.5%	70.0%	38.1%	
	Third pregnancy or more	No	0	0	2	15	4	21	
		%	.0%	.0%	5.0%	37.5%	10.0%	13.1%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	

GRAPH-2

DISTRIBUTION OF PATIENTS ACCORDING TO AGE AND RISK FACTOR



From the above table and graph 81% of mild pre eclampsia, 68.4% of severe pre eclampsia and 75% of eclampsia is seen in primigravida.70% of abruption is seen in 2nd gravida and more with P value at 1% level of significance.

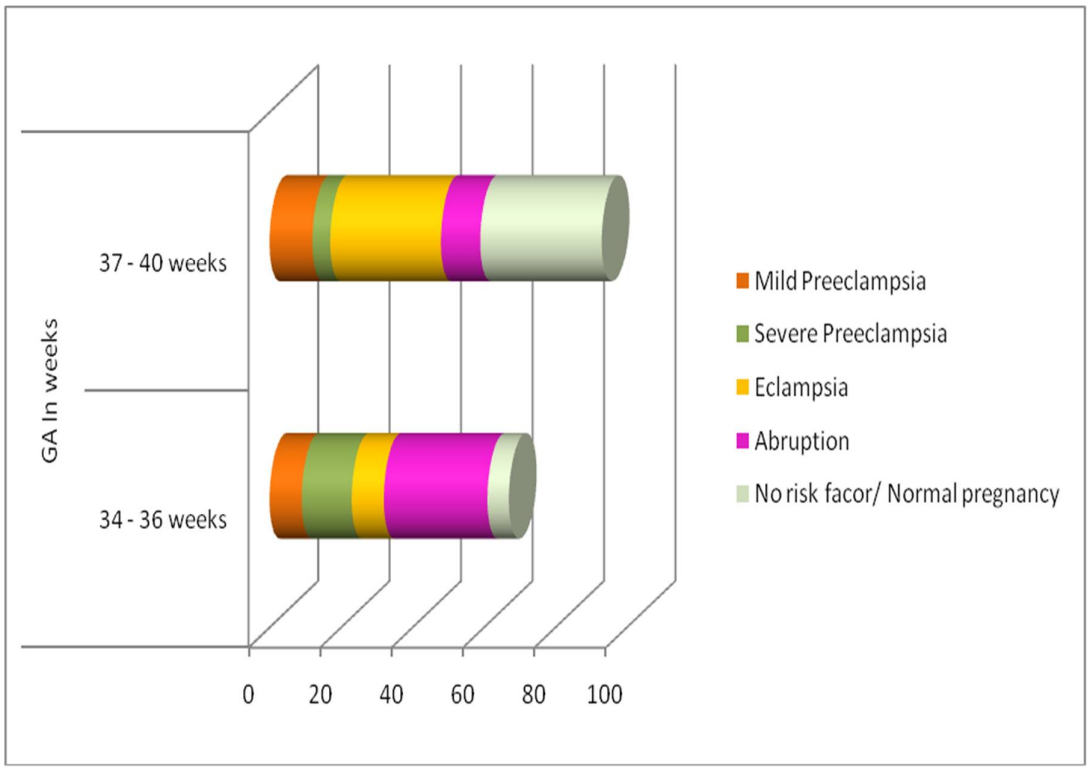
TABLE-13

DISTRIBUTION OF PATIENTS ACCORDING TO GESTATIONAL AGE IN WEEKS AND RISK FACTOR

			Risk Factor						P value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor/ Normal pregnancy	Total	
GA In weeks	34 - 36 weeks	No	9	14	9	29	6	67	0.000**
		%	42.9%	73.7%	22.5%	72.5%	15.0%	41.9%	
	37 - 40 weeks	No	12	5	31	11	34	93	
		%	57.1%	26.3%	77.5%	27.5%	85.0%	58.1%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0 %	

GRAPH-3

DISTRIBUTION OF PATIENTS ACCORDING TO GESTATIONAL AGE IN WEEKS AND RISK FACTOR



x-axis → number of patients.

y-axis→ gestational age in weeks.

p value at 1% level of significance.

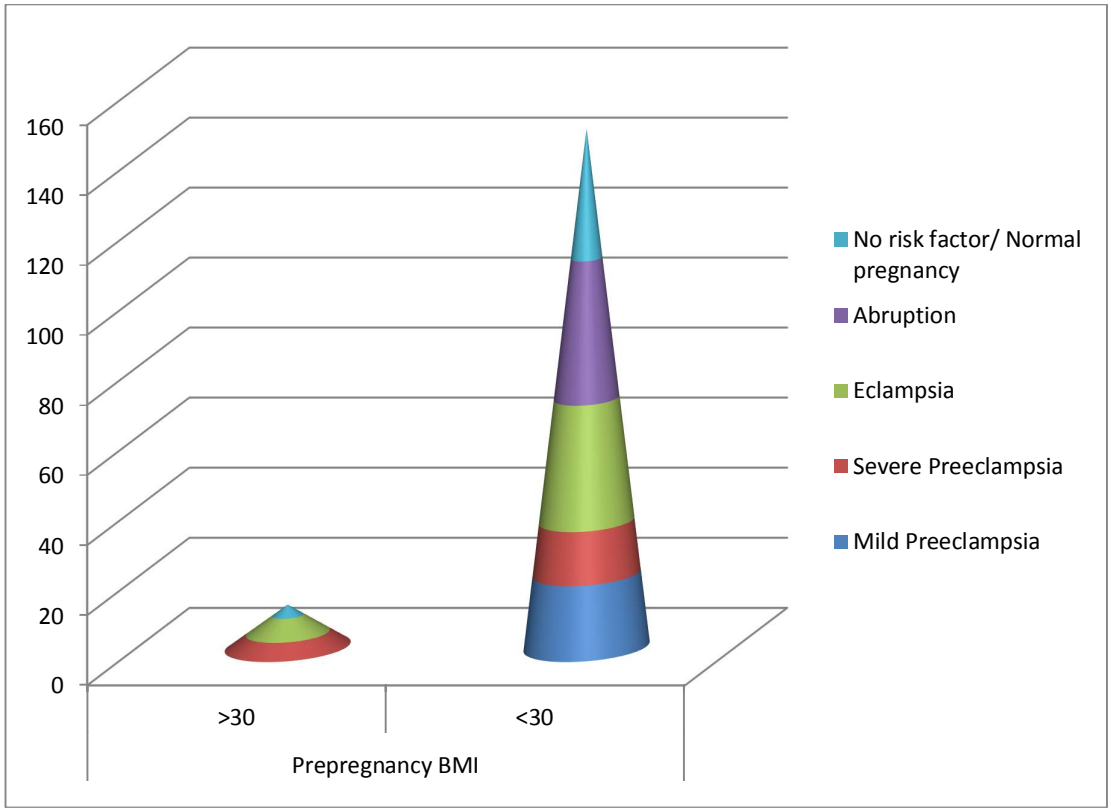
TABLE-14

DISTRIBUTION OF PATIENTS ACCORDING TO PRE PREGNANCY BMI AND RISK FACTOR

			Risk Factor					Total	P value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor / Normal pregnancy		
Pre pregnancy BMI	>30	No	0	4	5	0	3	12	0.022*
		%	.0%	21.1%	12.5%	.0%	7.5%	7.5%	
	<30	No	21	15	35	40	37	148	
		%	100.0%	78.9%	87.5%	100.0%	92.5%	92.5%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	

GRAPH-4

DISTRIBUTION OF PATIENTS ACCORDING TO PRE PREGNANCY BMI AND RISK FACTOR



x-axis→ BMI

y-axis→ number of patients

p value at 5% level of significance

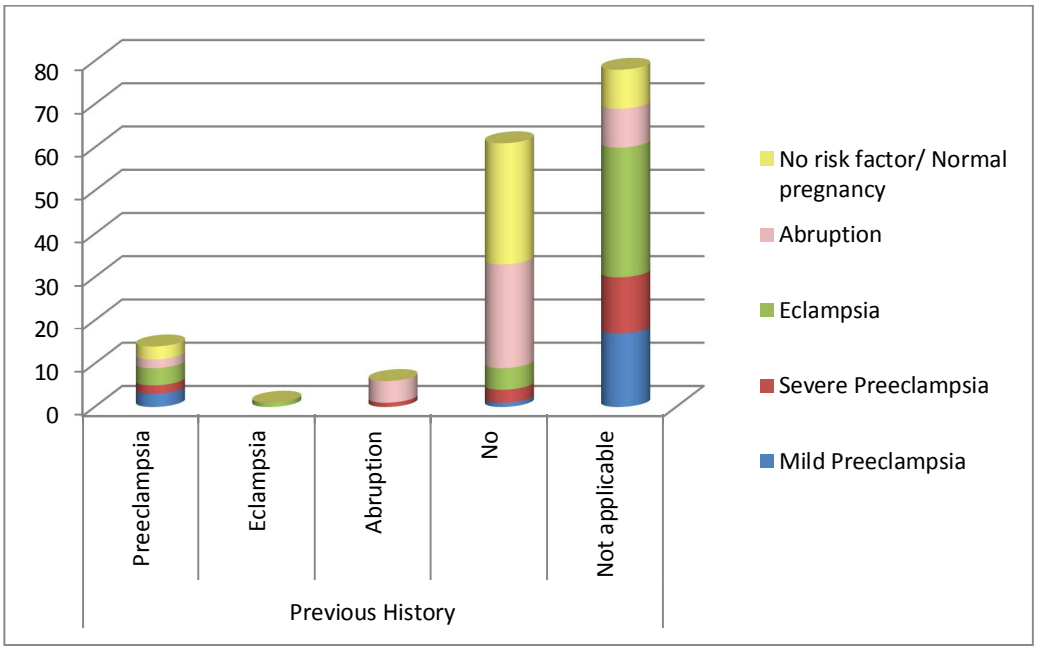
TABLE-15

DISTRIBUTION OF PATIENTS ACCORDING TO PREVIOUS HISTORY AND RISK FACTOR

			Risk Factor					Total	P Value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor/ Normal pregnancy		
Previous History	Preeclampsia	No	3	2	4	2	3	14	0.000
		%	21.4%	14.3%	28.6%	14.3%	21.4%	100.0%	
	Eclampsia	No	0	0	1	0	0	1	
		%	.0%	.0%	100.0%	.0%	.0%	100.0%	
	Abruption	No	0	1	0	5	0	6	
		%	.0%	16.7%	.0%	83.3%	.0%	100.0%	
	No	No	1	3	5	24	28	61	
		%	1.6%	4.9%	8.2%	39.3%	45.9%	100.0%	
	Not applicable	No	17	13	30	9	9	78	
		%	21.8%	16.7%	38.5%	11.5%	11.5%	100.0%	
Total		No	21	19	40	40	40	160	
		%	13.1%	11.9%	25.0%	25.0%	25.0%	100.0%	

GRAPH- 5

DISTRIBUTION OF PATIENTS ACCORDING TO PREVIOUS HISTORY AND RISK FACTOR



In this study 35.7% of pre eclamptics had past history of pre eclampsia, 100% of patients with past history of eclampsia developed eclampsia and 83.3% of patients developed abruption with p value at 1% level of significance.

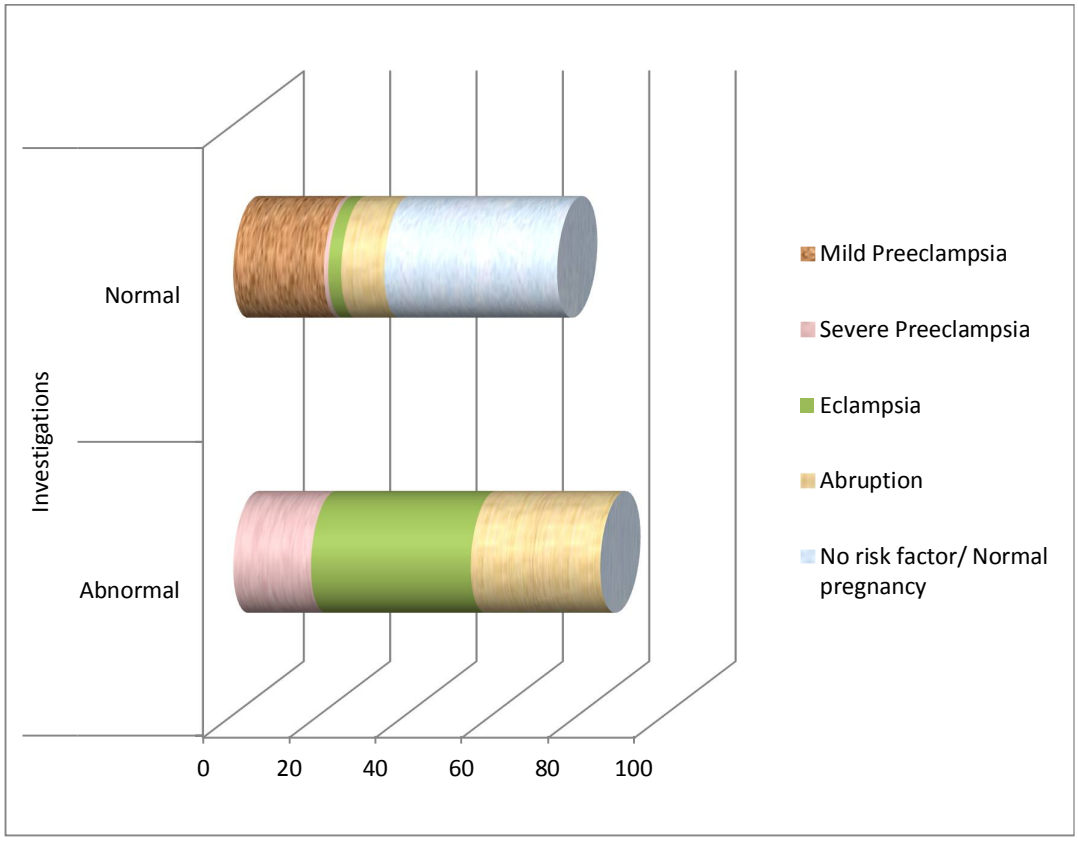
TABLE-16

DISTRIBUTION OF PATIENTS ACCORDING TO INVESTIGATIONS AND RISK FACTOR

			Risk Factor					Total	P value
			Mild Pre eclampsia	Severe Pre eclampsia	Eclampsia	Abruption	No risk factor/ Normal pregnancy		
Investigations	Abnormal	No	0	18	37	30	0	85	0.000**
		%	.0%	94.7%	92.5%	75.0%	.0%	53.1%	
	Normal	No	21	1	3	10	40	75	
		%	100.0%	5.3%	7.5%	25.0%	100.0%	46.9%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	

GRAPH-6

DISTRIBUTION OF PATIENTS ACCORDING TO INVESTIGATIONS AND RISK FACTOR



x-axis→number of patients

y-axis→investigations

p value at 1% level of significance

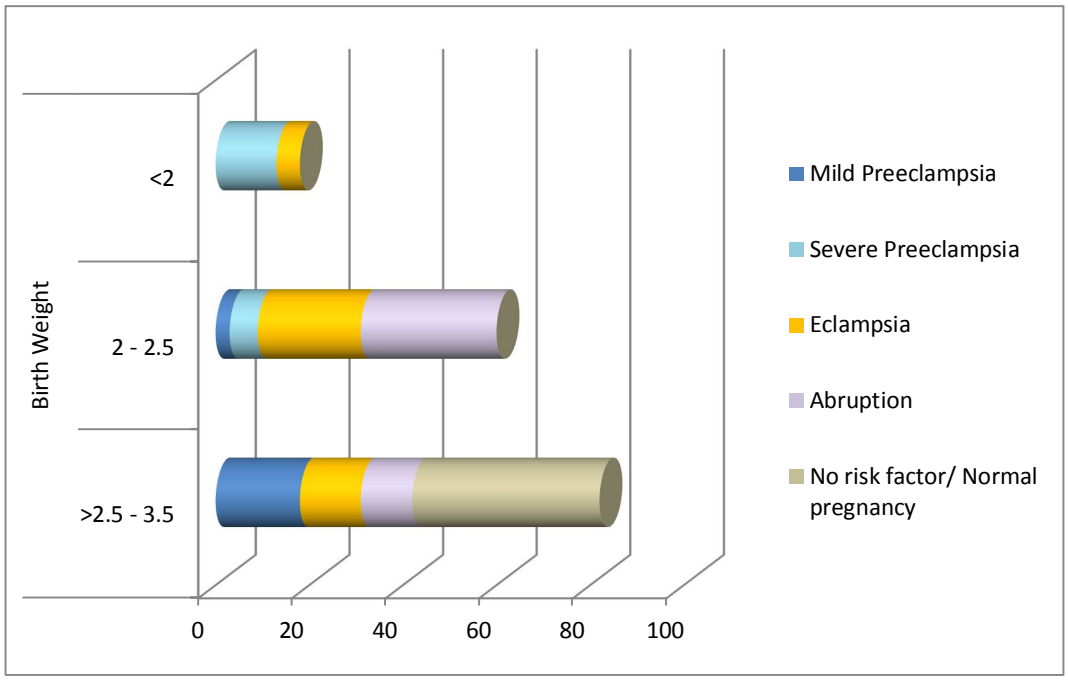
TABLE-17

DISTRIBUTION OF PATIENTS ACCORDING TO BIRTH WEIGHT AND RISK FACTOR

			Risk Factor						P value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor/ Normal pregnancy	Total	
Birth Weight	>2.5 - 3.5	No	18	0	13	11	40	82	0.000**
		%	85.7%	.0%	32.5%	27.5%	100.0%	51.2%	
	2 - 2.5	No	3	6	22	29	0	60	
		%	14.3%	31.6%	55.0%	72.5%	.0%	37.5%	
	<2	No	0	13	5	0	0	18	
		%	.0%	68.4%	12.5%	.0%	.0%	11.2%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0 %	

GRAPH 7

DISTRIBUTION OF PATIENTS ACCORDING TO BIRTH WEIGHT AND RISK FACTOR



x-axis→ number of patients

y-axis→ birth weight of babies

p value at 1% level of significance.

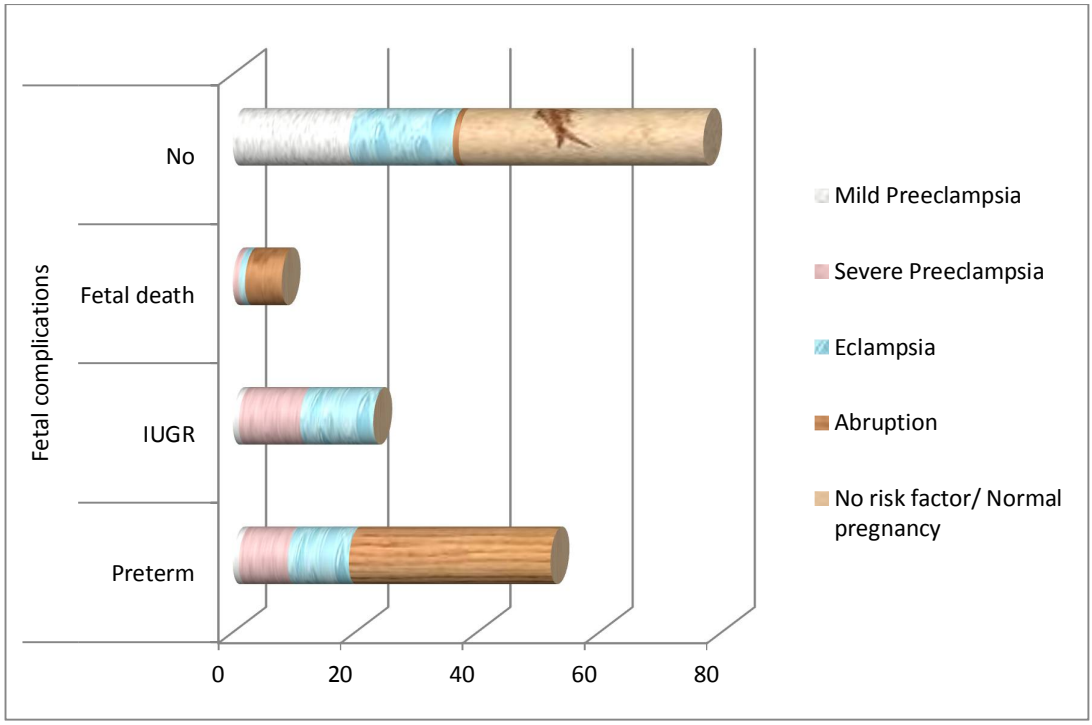
TABLE-18

DISTRIBUTION OF PATIENTS ACCORDING TO FETAL COMPLICATIONS AND RISK FACTOR

			Risk Factor					Total	P value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor/ Normal pregnancy		
Fetal complications	Preterm	No	1	8	10	33	0	52	0.000**
		%	4.8%	42.1%	25.0%	82.5%	.0%	32.5%	
	IUGR	No	1	10	12	0	0	23	
		%	4.8%	52.6%	30.0%	.0%	.0%	14.4%	
	Fetal death	No	0	1	1	6	0	8	
		%	.0%	5.3%	2.5%	15.0%	.0%	5.0%	
	No	No	19	0	17	1	40	77	
		%	90.5%	.0%	42.5%	2.5%	100.0%	48.1%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	

GRAPH-8

DISTRIBUTION OF PATIENTS ACCORDING TO FETAL COMPLICATIONS AND RISK FACTOR



In this study, 90.5 % of babies born to mild pre eclamptics are normal. 52.6% of babies born to severe pre eclamptics and 30% born to eclamptics have IUGR and 82.5% babies born to patients with abruption are preterm with p value at 1% level of significance.

TABLE-19

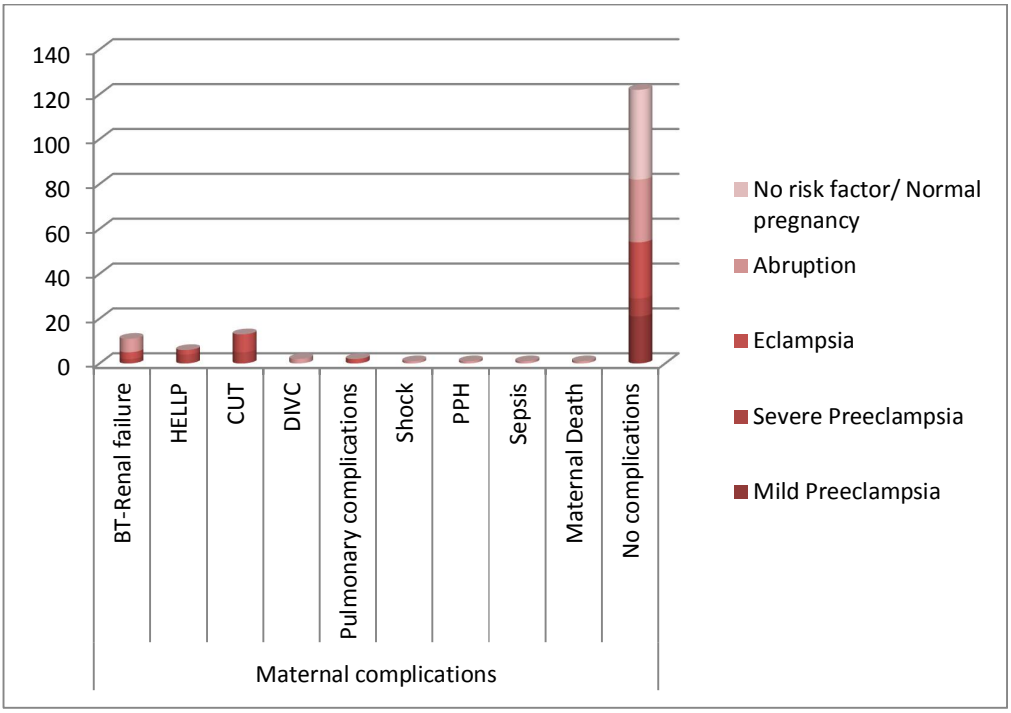
DISTRIBUTION OF PATIENTS ACCORDING TO MATERNAL COMPLICATIONS AND RISK FACTOR

			Risk Factor					Total	P value
			Mild Preeclampsia	Severe Preeclampsia	Eclampsia	Abruption	No risk factor/		
Maternal complications	Renal failure	No	0	2	3	6	0	11	0.000**
		%	.0%	10.5%	7.5%	15.0%	.0%	6.9%	
	HELLP	No	0	4	2	0	0	6	
		%	.0%	21.1%	5.0%	.0%	.0%	3.8%	
	CVT	No	0	5	8	0	0	13	
		%	.0%	26.3%	20.0%	.0%	.0%	8.1%	
	DIVC	No	0	0	0	2	0	2	
		%	.0%	.0%	.0%	5.0%	.0%	1.2%	
	Pulmonary complications	No	0	0	2	0	0	2	
		%	.0%	.0%	5.0%	.0%	.0%	1.2%	

	Shock	No	0	0	0	1	0	1	
		%	.0%	.0%	.0%	2.5%	.0%	.6%	
	PPH	No	0	0	0	1	0	1	
		%	.0%	.0%	.0%	2.5%	.0%	.6%	
	Sepsis	No	0	0	0	1	0	1	
		%	.0%	.0%	.0%	2.5%	.0%	.6%	
	Maternal Death	No	0	0	0	1	0	1	
		%	.0%	.0%	.0%	2.5%	.0%	.6%	
	No complications	No	21	8	25	28	40	122	
		%	100.0%	42.1%	62.5%	70.0%	100.0%	76.2%	
Total		No	21	19	40	40	40	160	
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	

GRAPH-9

DISTRIBUTION OF PATIENTS ACCORDING TO MATERNAL COMPLICATIONS AND RISK FACTOR



In this study mild pre eclamptics did not develop any complications. CVT is seen in 26.3% of severe pre eclamptics and 20% of eclamptics. HELLP syndrome is seen in 21.1% of severe pre eclamptics. Renal failure developed in 15% of patients with abruption and 10.5% of patients with severe pre eclampsia. DIVC is seen in 5% of patients with abruption and maternal death occurred in 2.5% of abruptions.

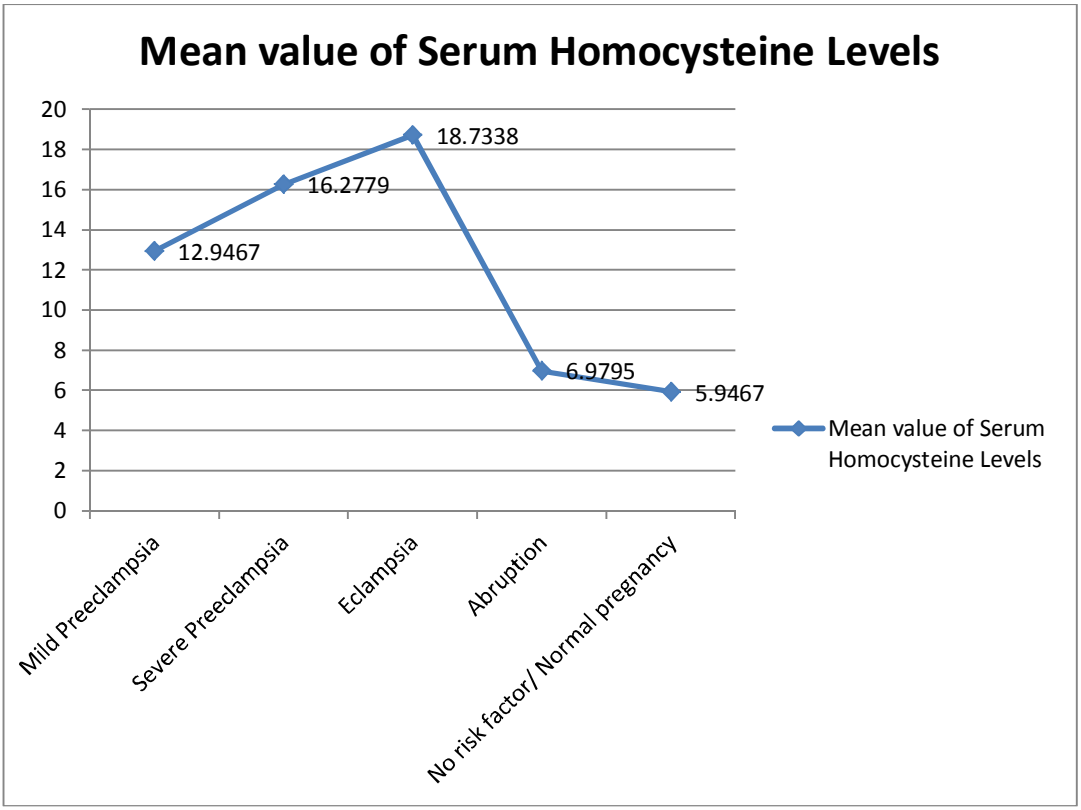
TABLE-20

DISTRIBUTION OF PATIENTS ACCORDING TO SERUM HOMOCYSTEINE LEVELS AND RISK FACTOR

Risk Factor	Mean	N	Std. Deviation	P value
Mild Preeclampsia	12.9467	21	1.45837	0.000**
Severe Preeclampsia	16.2779	19	.52248	
Eclampsia	18.7338	40	1.61880	
Abruption	6.9795	40	.71221	
No risk factor/ Normal pregnancy	5.9467	40	.41856	
Total	11.5472	160	5.49306	

GRAPH-10

DISTRIBUTION OF PATIENTS ACCORDING TO SERUM HOMOCYSTEINE LEVELS AND RISK FACTOR



X-axis→ risk factor

Y-axis→ mean value of serum homocysteine levels

P value at 1% level of significance.

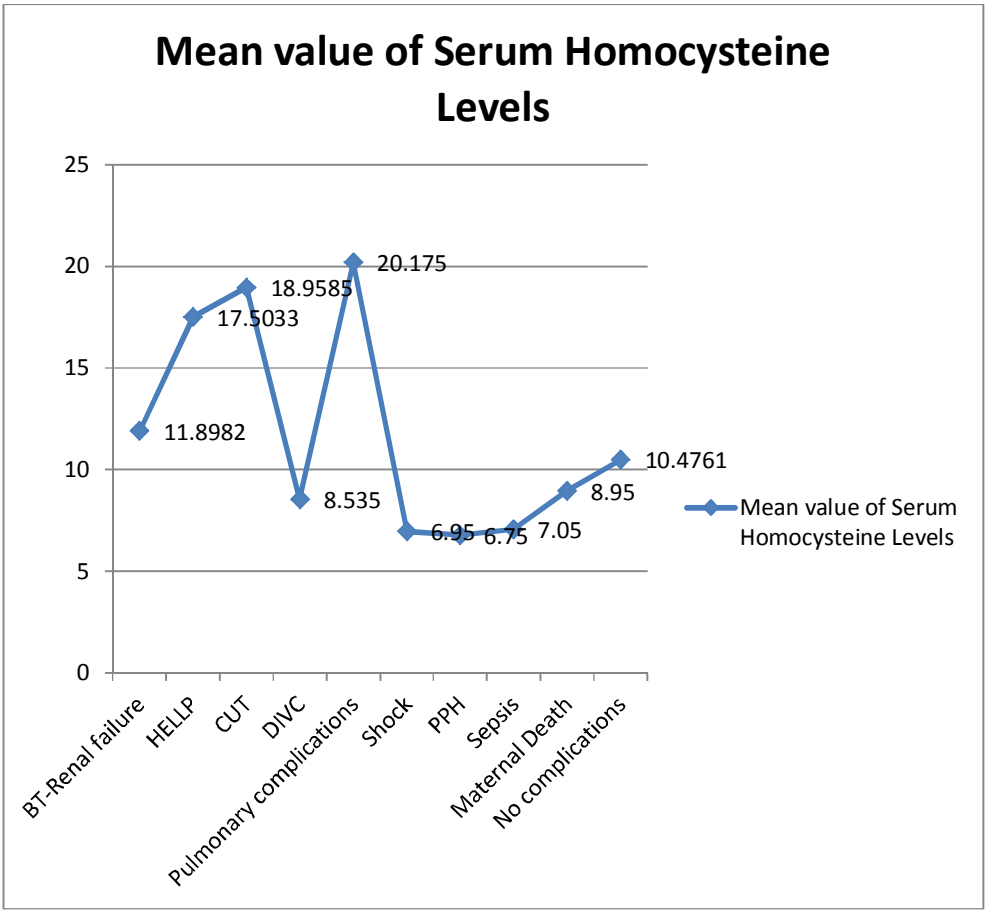
TABLE-21

DISTRIBUTION OF PATIENTS ACCORDING TO SERUM HOMOCYSTEINE LEVELS AND MATERNAL COMPLICATIONS

Maternal complications	Mean	N	Std. Deviation	P value
Renal failure	11.8982	11	6.10805	0.000**
HELLP	17.5033	6	2.06152	
CVT	18.9585	13	2.19650	
DIVC	8.5350	2	.07778	
Pulmonary complications	20.1750	2	2.62337	
Shock	6.9500	1	.	
PPH	6.7500	1	.	
Sepsis	7.0500	1	.	
Maternal Death	8.9500	1	.	
No complications	10.4761	122	5.00589	
Total	11.5472	160	5.49306	

GRAPH-11

DISTRIBUTION OF PATIENTS ACCORDING TO SERUM HOMOCYSTEINE LEVELS AND MATERNAL COMPLICATIONS



X-axis→maternal complications

Y-axis →mean serum homocysteine levels

DISCUSSION

Importance of serum homocysteine levels has been described in 1960s by Dr.Kilmer McCully. Increased homocysteine levels is a risk factor for endothelial dysfunction and vascular disease.

In pregnant woman, increased homocysteine level is likely to injure the vascular endothelium. Vascular changes caused by homocysteine are similar to that seen in preeclampsia and eclampsia.

The association between increased levels of homocysteine and preeclampsia has been suggested by Dekker et al[81]. The relationship has been shown in early pregnancy by Cotter et al[82],2nd trimester by Sorensen et al[83] and 3rd trimester by Sanchez et al[84].

In our study maximum number of patients belong to 20-30 years of age(73.8%).In the study done by Mozammel Hoque et al,all patients were in 20-30 years of age and in study done by Qureshi et al majority of patients belonged to 20-30 years of age.

In our study, 48.8% of patients are primigravida and all patients belong to 34-40 weeks of gestation. In study done by Qureshi et al 32 -40 weeks of gestational age was included.

In our study, the severity of preeclampsia is directly related to hyper homocysteinemia with mean homocysteine levels being 12.94

micro mol/L in mild preeclampsia,16.2 micro mol/L in severe preeclampsia and 5.94 micro mol/L in normal pregnancies with p value being 0.000 ,similar to study of Khosrowbeygi et al[85] where the homocystiene levels are 11.49 +/- 1.19 micro mol/L in mild preeclampsia , 17.4+/- 2.7 micro mol /L in severe preeclampsia and 6.38 +/- 0.3 micro mol /L in normal pregnancies with p value 0.000.

In our study homocysteine levels are increased in pre eclampsia and eclampsia but levels in eclampsia are higher (18.7 micro mol/L) than in severe preeclampsia(16.2 micro mol/L) similar to Mozammel Hoque et al where levels in eclampsia are 10.57 +/- 3.39 micro mol /L and in pre eclampsia it is 9.54 +/- 3.21 micro mol /L.

Our study does not show an association between homocysteine levels and placental abruption similar to studies of Qureshi et al and Steegeres et al(52).

Our study shows an association between increased homocysteine levels and low birth weight with p value being 0.000 similar to studies of Qureshi et al and Murphy et al(86) with p value being less than 0.001.

	Mozammel Hoque et al		Qureshi et al		Our study	
Age	20-30 yrs		22-32 yrs		20-35 yrs	
Gestational age	26-34 weeks		32-40 weeks		34-40 weeks	
	Patient count	Serum Homocyste-ine levels (Micromoles per litre)	Patient count	Serum Homocyste-ine levels (Micromoles per litre)	Patient count	Serum Homo-cysteine levels (Mi-cromoles per litre)
Normal pregnancy	136	6.86±2.47	112	8.19±3.05	40	5.94
Preeclamsia	84	9.54±3.21	61	9.42±3.91	80	16.20
Eclampsia	120	10.57±3.39	49	10.07±7.71	40	18.70
Abruption			110	7.83±4.65	40	6.97
P value	0.000		0.01		0.000	

From the above study , it is seen that measuring homocysteine levels during pregnancy will help us to predict the development of preeclampsia,eclampsia and abruption.

SUMMARY

A study is made on 160 antenatal patients who attended the obstetrics and gynaecology department in Coimbatore medical college to know the levels of serum homocysteine in preeclampsia, eclampsia , abruption and normal pregnancies and whether the levels were indicators of severity over a period of one year.

- In our study, preeclampsia and eclampsia is common in primigravida.
- 62.5 % of abruptions occured with advancing age of mother and more common in second gravida or more.
- Mild pre eclamptics did not develop any complications. CVT is more commonly seen in severe preeclampsia (26.3%) and eclampsia (20%).
- Growth restriction of fetuses is more common with severe preeclampsia (52.6%) and preterm babies is common with abruption (82.5%)
- Serum homocysteine levels are indicators of severity in pre eclampsia and eclampsia but there was no association with abruption with p value at 1% level of significance.

- Our study involves small population and hence association between Serum homocysteine and abruptio placenta is not proved.
- Studies have supported the evidence of lowering Serum homocysteine levels by supplementing vitamin B and folic acid.
- Hence supplementation of folic acid and B complex vitamins from first trimester of pregnancy can be useful in reducing serum homocysteine levels thereby preventing preeclampsia, eclampsia, abruption and its complications which is yet to be proved by further studies.

CONCLUSION

- Measurement of serum homocysteine levels can be included in routine ante natal care management in the hospital.
- Severity of the disease can be interpreted by the levels of homocysteine.
- Homocysteine levels can be lowered by supplementation with folic acid and other B complex vitamins as supported by studies in review of literature, there by preventing maternal and fetal complications.
- Based on these studies, in a low resource setting and developing country like India, to be cost effective, B complex vitamins along with peri conceptional folic acid supplementation from early pregnancy can be very useful to prevent hyperhomocysteinemia and related diseases like preeclampsia , eclampsia and abruption and its complications there by reducing maternofetal morbidity and mortality.

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ANNEXURE 1

PROFORMA

Name : Age : IP NO :

Address :

Parity :

LMP : EDD :

Chief complaints :

Menstrual history :

Marital history :

Obstetric history :

History of pre eclampsia,eclampsia and abruption in previous pregnancy is asked for.

Past history :

Associated risk factors

Family history :

Personal history :

Dietary history

Treatment taken

Examination of patient :

General condition :

Anaemia/pedal edema/clubbing/lymphadenopathy/nutritional status

Pulse rate :

Blood pressure :

CVS :

RS :

Per abdomen :

Per vaginal :

Investigations :

Blood grouping & typing

Haemoglobin

Platelet count

Urine albumin

Sugar

HIV

HbsAg

VDRL

Blood sugar

Special investigations

Blood urea

Serum creatinine

LFT

Bleeding time

Clotting time

PT,aPTT

Serum uric acid

Serum homocysteine levels

Ultrasound

CT brain if needed

ANNEXURE -2

CONSENT FORM

Yourself Mr./Mrs./Ms…….. are being asked to be a participant in the research study titled **" A COMPARATIVE ANALYSIS OF SERUM HOMOCYSTEINE LEVELS IN PRE ECLAMPSIA, ECLAMPSIA, ABRUPTIO PLACENTA AND NORMAL PREGNANCIES"** in CMC Hospital, Coimbatore, conducted by **DR. V.K.T ANNURADHA**, Post Graduate Student, Department of **OBSTETRICS AND GYNAECOLOGY**, Coimbatore Medical College. You are eligible after looking into the inclusion criteria. You can ask any question you may have before agreeing to participate.

Purpose of Research

- To study the relationship between the levels of serum homocysteine in normal pregnancy and pregnancies complicated by pre eclampsia , eclampsia and abruption.
- To know, if the levels of serum homocysteine are indicators of severity of pre eclampsia, eclampsia and abruption, to reduce maternofetal morbidity and mortality.

Decline from Participation

You have the option to decline from participation in the study existing protocol for your condition.

Privacy and Confidentiality

Privacy of individuals will be respected and any information about you or provided by you during the study will be kept strictly confidential.

Authorization to publish Results

Results of the study may be published for scientific purposes and/or presented to scientific groups, however you will not be identified.

Statement of Consent

I volunteer and consent to participate in this study. I have read the consent or it has been read to me. The study has been fully explained to me, and I may ask questions at any time.

---------- ----------

Signature /Left thumb impression Date

(volunteer)

---------- ----------

Signature of witness Date

ஒப்புதல் படிவம்

பெயர் -

பாலினம் -

வயது -

முகவரி -

அரசு கோவை மருத்துவக்கல்லூரியில் மகப்பேறு மருத்துவ துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவி அனுராதா வி.கே.டி அவர்கள் மேற்கொள்ளும் ஹோமோசிஸ்டின் என்னும் புரதத்தின் தன்மையை மகப்பேறு காலத்தில் இரத்தத்தில் அளவிட்டு மேலும் அதனால் எற்படும் பின்விளைவுகளை பற்றியும் ஆய்வில் செய்முறை மற்றும் அனைத்து விவரங்களையும் கேட்டு கொண்டு எனது சந்தேகங்களை தெளிவுப்படுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

நான் இந்த ஆய்வில் முழு சம்மதத்துடன், சுய சிந்தனையுடனும் கலந்து கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் என்னுடைய அனைத்து விபரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதை தெரிவித்துக் கொள்கிறேன். எந்த நேரத்திலும் இந்த ஆய்விலிருந்து நான் விலகிக் கொள்ள எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இப்படிக்கு

KEY TO MASTER CHART

Age :

21-30 yrs	-	1
31-35 yrs	-	2

Parity :

First pregnancy	-	1
Second pregnancy	-	2
Third pregnancy	-	3
Or more		

GA in weeks :

34-36 weeks	-	1
37-40 weeks	-	2

Pre pregnancy BMI :

>30	-	1
<30	-	2

Previous h/o :

preeclampsia	-	1
Eclampsia	-	2
Abruption	-	3
No risk factors	-	4
Not applicable	-	5

Risk factors :

Mild preeclampsia	-	1
Severe preeclampsia	-	2
Eclampsia	-	3
Abruption	-	4

Normal pregnancy	-	5
Investigations :		
Abnormal	-	1
Normal	-	2
Maternal complications :		
Renal failure	-	1
HELLP	-	2
CVT	-	3
DIVC	-	4
Pulmonary complication	-	5
Shock	-	6
PPH	-	7
Sepsis	-	8
Maternal death	-	9
No complications	-	10
Birth weight :		
2.5 kg – 3.5 kg	-	1
2.0kg - 2.5 kg	-	2
< 2.0 kg	-	3
Fetal complications :		
Preterm	-	1
IUGR	-	2
Fetal death	-	3
Congenital malformations	-	4
No complications	-	5

MASTER CHART

S.No	I.P No	Age	Parity	GA In weeks	Prepregnancy BMI	Previous History	Risk Factor	Investigations	Serum Homocysteine Levels	Maternal complications	Birth Weight	Fetal complications
1	259256	1	2	2	2	4	5	2	6.19	10	1	5
2	319258	1	2	2	2	4	5	2	6.25	10	1	5
3	281963	1	2	2	2	4	5	2	6.03	10	1	5
4	963825	1	1	2	2	5	5	2	5.18	10	1	5
5	754632	1	1	2	2	5	5	2	5.75	10	1	5
6	842425	1	1	2	2	5	5	2	5.26	10	1	5
7	132854	1	1	2	2	5	5	2	5.13	10	1	5
8	263859	1	1	2	2	5	5	2	6.08	10	1	5
9	748563	1	2	2	2	4	5	2	5.9	10	1	5
10	825714	1	2	2	2	4	5	2	5.81	10	1	5
11	109574	1	2	2	2	4	5	2	5.67	10	1	5
12	857496	1	2	2	2	4	5	2	6.42	10	1	5
13	235689	1	2	2	2	4	5	2	6.1	10	1	5
14	135287	1	2	2	2	4	5	2	6.32	10	1	5
15	745698	1	2	2	2	4	5	2	6.03	10	1	5
16	896475	1	2	2	2	4	5	2	5.62	10	1	5
17	365869	1	2	2	2	4	5	2	5.89	10	1	5
18	724965	1	2	2	2	4	5	2	5.54	10	1	5
19	123765	1	2	2	2	4	5	2	5.38	10	1	5
20	845936	1	2	2	2	4	5	2	5.04	10	1	5
21	759462	1	2	2	2	4	5	2	6.18	10	1	5
22	798465	1	2	2	2	4	5	2	6.22	10	1	5

23	253625	1	2	2	2	4	5	2	6.98	10	1	5
24	402635	1	2	2	2	4	5	2	6.52	10	1	5
25	256398	1	3	2	2	1	5	2	6.48	10	1	5
26	865945	1	3	1	2	1	5	2	6.33	10	1	5
27	125874	1	2	1	2	1	5	2	6.27	10	1	5
28	746369	1	2	1	2	4	5	2	5.99	10	1	5
29	124658	1	2	1	2	4	5	2	5.87	10	1	5
30	123458	1	1	2	1	5	5	2	5.65	10	1	5
31	875846	1	1	1	1	5	5	2	6.24	10	1	5
32	457865	1	1	2	1	5	5	2	6.01	10	1	5
33	235985	1	3	1	2	4	5	2	5.93	10	1	5
34	956582	1	3	2	2	4	5	2	6.14	10	1	5
35	457865	1	2	2	2	4	5	2	6.25	10	1	5
36	978758	1	1	1	2	5	1	2	11.01	10	1	5
37	484444	1	1	1	2	5	1	2	11.96	10	1	5
38	936784	1	1	1	2	5	1	2	12.02	10	1	5
39	123854	1	1	1	2	5	1	2	12.26	10	1	5
40	963748	1	1	1	2	5	1	2	11.99	10	2	1
41	741852	1	1	1	2	5	1	2	12.04	10	1	5
42	963825	1	1	1	2	5	1	2	13.05	10	1	5
43	662513	1	1	1	2	5	1	2	13.48	10	1	5
44	885596	1	1	1	2	5	1	2	13.56	10	1	5
45	768254	1	1	2	2	5	1	2	13.64	10	1	5
46	142578	2	1	2	2	5	1	2	13.41	10	1	5
47	325566	2	1	2	2	5	1	2	12.98	10	1	5
48	123450	2	1	2	2	5	1	2	12.54	10	1	5
49	321856	1	2	2	2	1	1	2	12.67	10	1	5
50	404044	1	1	2	2	5	1	2	13.12	10	1	5
51	213854	2	1	2	2	5	1	2	13.44	10	1	5

52	525657	2	1	2	2	5	1	2	13.65	10	1	5
53	504058	2	1	2	2	5	1	2	14.05	10	2	2
54	779856	2	1	1	1	5	2	1	15.06	10	3	1
55	701548	1	1	1	1	5	2	1	15.98	2	3	2
56	632109	2	1	1	1	5	2	1	15.91	10	3	2
57	418529	1	1	1	1	5	2	2	15.97	10	3	2
58	101891	1	1	1	2	5	2	1	16.04	3	3	1
59	907525	1	1	1	2	5	2	1	16.22	10	3	1
60	785629	1	1	1	2	5	2	1	16.85	3	3	1
61	801852	1	1	1	2	5	2	1	16.49	10	3	2
62	665425	1	1	1	2	5	2	1	16.84	2	3	3
63	415028	1	1	1	2	5	2	1	16.87	1	3	1
64	102856	1	1	1	2	5	2	1	15.97	10	3	1
65	449286	1	1	1	2	5	2	1	16.54	3	3	1
66	854769	1	1	2	2	5	2	1	15.55	10	2	2
67	754863	1	2	2	2	1	2	1	17.01	3	2	2
68	824225	1	2	1	2	1	2	1	16.54	1	3	1
69	708465	1	2	2	2	4	2	1	16.96	3	2	2
70	614253	1	2	2	2	4	2	1	16.53	10	2	2
71	348569	1	1	2	2	5	3	1	20.02	3	2	2
72	475685	1	1	2	2	5	3	1	17.01	10	1	5
73	602514	1	1	2	2	5	3	1	18.91	3	1	5
74	512865	1	1	2	2	5	3	1	17.89	3	1	5
75	321301	1	1	2	2	5	3	1	17.77	10	1	5
76	623019	1	1	2	2	5	3	1	17.99	1	1	5
77	552657	1	1	2	2	5	3	1	16.52	10	1	5
78	440444	1	1	2	2	5	3	1	17.03	10	1	5
79	895956	1	1	2	2	5	3	1	15.92	10	1	5
80	594565	1	1	2	2	5	3	1	18.06	10	1	5

81	857486	1	1	2	2	5	3	1	16.7	10	1	5
82	501807	1	1	2	2	5	3	1	19.33	1	1	3
83	732185	1	1	2	2	5	3	1	18.32	5	2	2
84	882898	2	1	2	2	5	3	1	17.41	10	2	2
85	613425	2	1	2	2	5	3	1	16.91	10	2	2
86	141415	2	1	2	2	5	3	1	17.99	10	2	2
87	701526	2	1	2	2	5	3	1	16.99	10	2	2
88	825936	2	1	2	2	5	3	1	20.31	2	1	5
89	789456	2	1	2	2	5	3	1	19.94	2	2	2
90	795546	2	1	2	2	5	3	1	17.96	10	2	5
91	844225	2	1	2	2	5	3	1	18.02	10	2	5
92	827514	1	1	2	2	5	3	1	18	10	2	5
93	764396	1	1	2	2	5	3	1	17.96	10	2	5
94	676582	2	1	2	2	5	3	1	17.89	10	2	5
95	501256	1	1	2	2	5	3	1	18.05	10	2	5
96	765095	1	2	2	2	1	3	1	19.06	10	2	1
97	623109	1	2	2	2	2	3	1	19.33	10	2	2
98	448324	1	2	2	2	1	3	1	18.64	10	1	2
99	741852	1	2	2	2	1	3	1	17.59	10	2	2
100	552658	1	2	2	2	4	3	1	18	10	2	2
101	500165	1	2	1	1	4	3	1	20.22	1	3	1
102	701508	1	2	1	1	4	3	1	21.34	3	3	1
103	805092	1	2	1	1	4	3	1	21.14	3	3	1
104	916852	1	3	1	2	1	3	1	20.26	3	3	1
105	874856	1	3	1	2	4	3	1	19.45	10	3	1
106	970255	2	2	1	2	4	4	2	7.52	10	2	1
107	789224	2	2	1	2	4	4	2	7.99	10	2	1
108	932784	2	2	1	2	4	4	2	7.25	10	2	1
109	741802	2	2	1	2	4	4	2	7.39	10	2	1

110	875486	2	2	1	2	4	4	2	6.18	10	2	1
111	412348	2	2	1	2	4	4	2	6.23	10	2	1
112	950011	2	2	1	2	4	4	2	6.95	10	2	1
113	801218	2	2	1	2	4	4	1	6.49	10	2	1
114	770154	2	2	1	2	4	4	1	6.28	10	2	1
115	791072	2	2	1	2	4	4	1	7.01	10	2	1
116	838659	2	2	1	2	4	4	1	7.55	10	2	1
117	876895	2	3	1	2	4	4	1	8.48	4	2	3
118	928659	2	3	1	2	4	4	1	8.59	4	2	3
119	707514	2	3	1	2	4	4	1	8.95	9	2	3
120	842684	2	3	1	2	4	4	1	7.51	10	2	3
121	751751	2	3	1	2	4	4	1	6.82	10	2	3
122	845865	2	3	1	2	4	4	1	6.75	10	2	3
123	897867	2	3	2	2	4	4	1	6.72	10	1	1
124	854847	2	3	2	2	4	4	1	6.35	10	1	1
125	799999	2	3	2	2	4	4	1	6.92	10	1	1
126	821614	2	3	2	2	4	4	1	7.89	10	1	1
127	948964	2	3	2	2	4	4	1	7.26	10	1	1
128	886688	2	3	2	2	4	4	1	6.22	10	1	1
129	893865	2	3	2	2	3	4	1	6.45	1	1	1
130	901542	2	1	2	2	4	4	1	6.56	1	1	1
131	912865	1	1	2	2	5	4	1	6.12	10	1	1
132	955873	1	1	1	2	5	4	1	6.17	10	1	1
133	937542	1	1	1	2	5	4	1	6.65	1	1	1
134	960459	1	1	1	2	5	4	1	6.89	1	2	1
135	922523	1	2	1	2	1	4	1	6.95	6	2	1
136	964825	1	2	1	2	1	4	1	6.75	7	2	1
137	994201	1	2	1	2	3	4	1	7.36	10	2	1
138	896523	1	2	1	2	3	4	1	6.27	10	2	1

139	878658	1	3	1	2	3	4	1	6.25	10	2	1
140	831259	1	3	1	2	3	4	1	7.05	8	2	1
141	257489	1	2	2	2	4	5	2	6.12	10	1	5
142	384569	1	2	2	2	4	5	2	6.18	10	1	5
143	687412	1	2	2	2	4	5	2	5.98	10	1	5
144	695412	1	2	2	2	4	5	2	5.29	10	1	5
145	787573	1	2	2	2	4	5	2	5.65	10	1	5
146	848280	1	2	2	2	1	1	2	9.23	10	1	5
147	664259	1	2	2	2	1	1	2	15.96	10	1	5
148	489426	1	2	2	2	4	1	2	15.82	10	2	5
149	284596	1	2	2	2	4	2	1	15.94	2	2	2
150	747673	1	2	1	2	3	2	1	16.01	2	2	2
151	778498	1	1	1	2	5	3	1	18.33	10	2	1
152	845896	1	1	1	1	5	3	1	20.94	3	2	1
153	321456	1	1	1	1	5	3	2	22.03	5	2	1
154	654123	1	1	1	2	5	3	2	22.56	3	2	1
155	258369	1	1	2	2	5	3	2	21.56	10	2	2
156	963852	1	1	1	2	5	4	2	6.12	10	2	1
157	654321	1	1	1	2	5	4	2	6.4	1	2	1
158	558488	1	1	1	2	5	4	2	6.98	1	2	1
159	947151	1	1	2	2	5	4	1	7.14	10	2	1
160	677485	1	1	2	2	5	4	1	7.77	10	2	5